FIG. 1

PhenoSense™ HIV Resistance Test Vector.

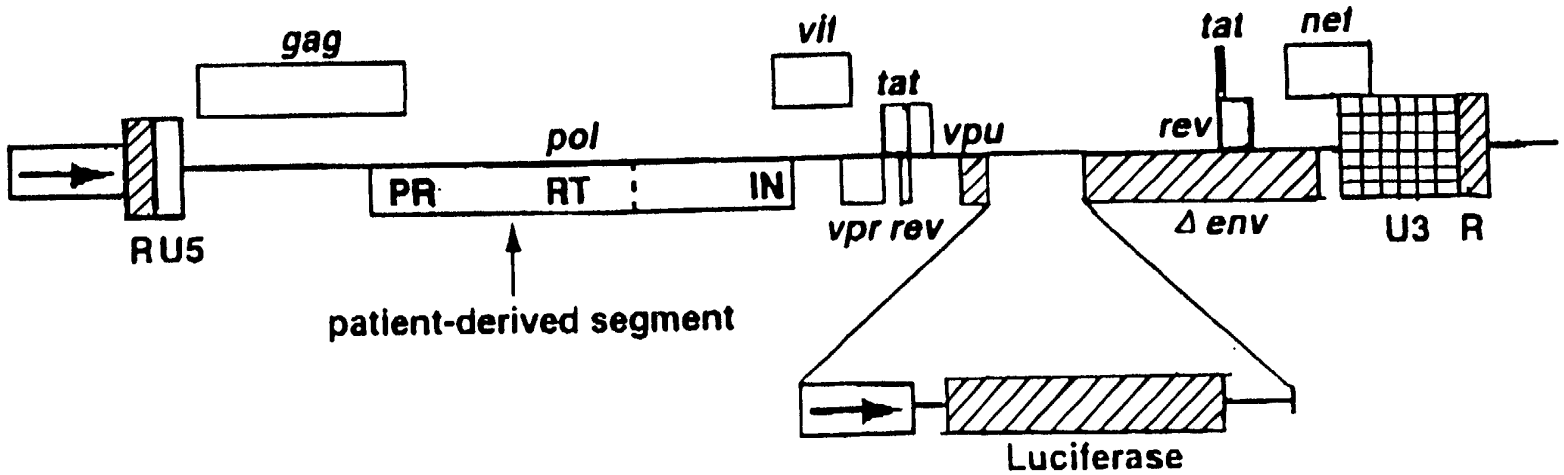


FIG. 2

PhenoSense™ HIV Schematic Diagram.

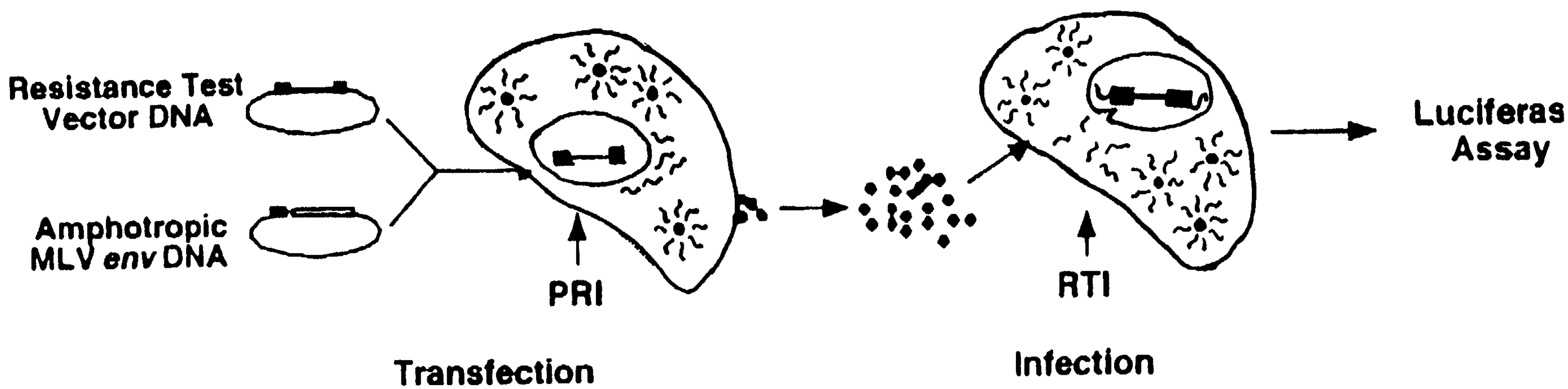
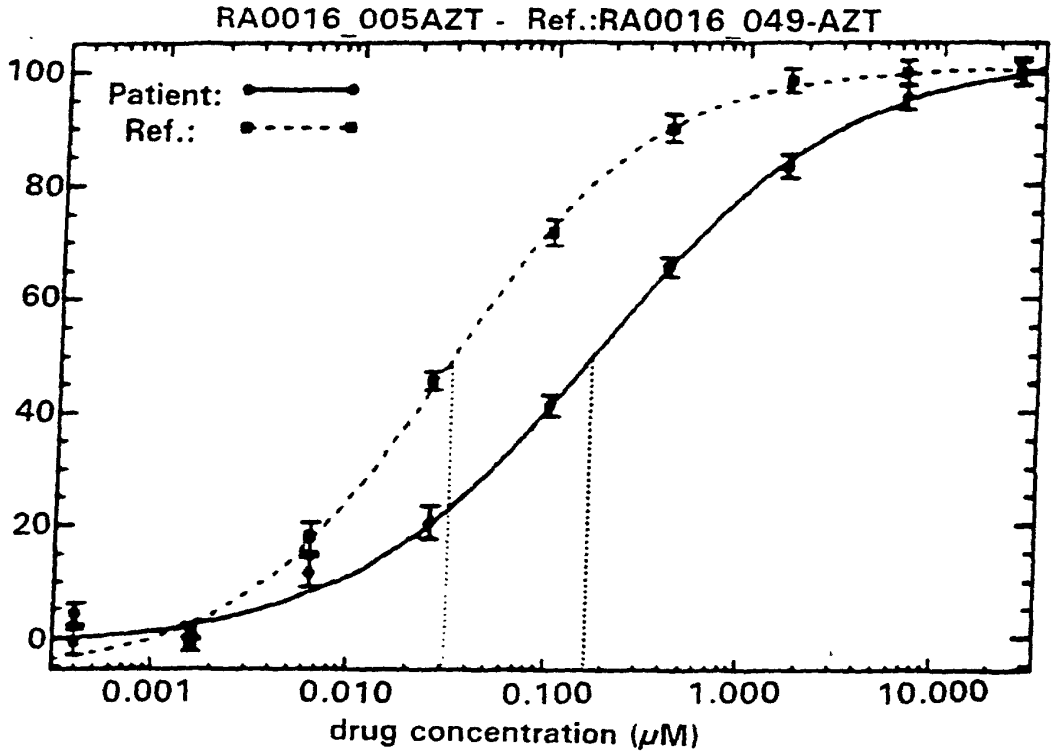


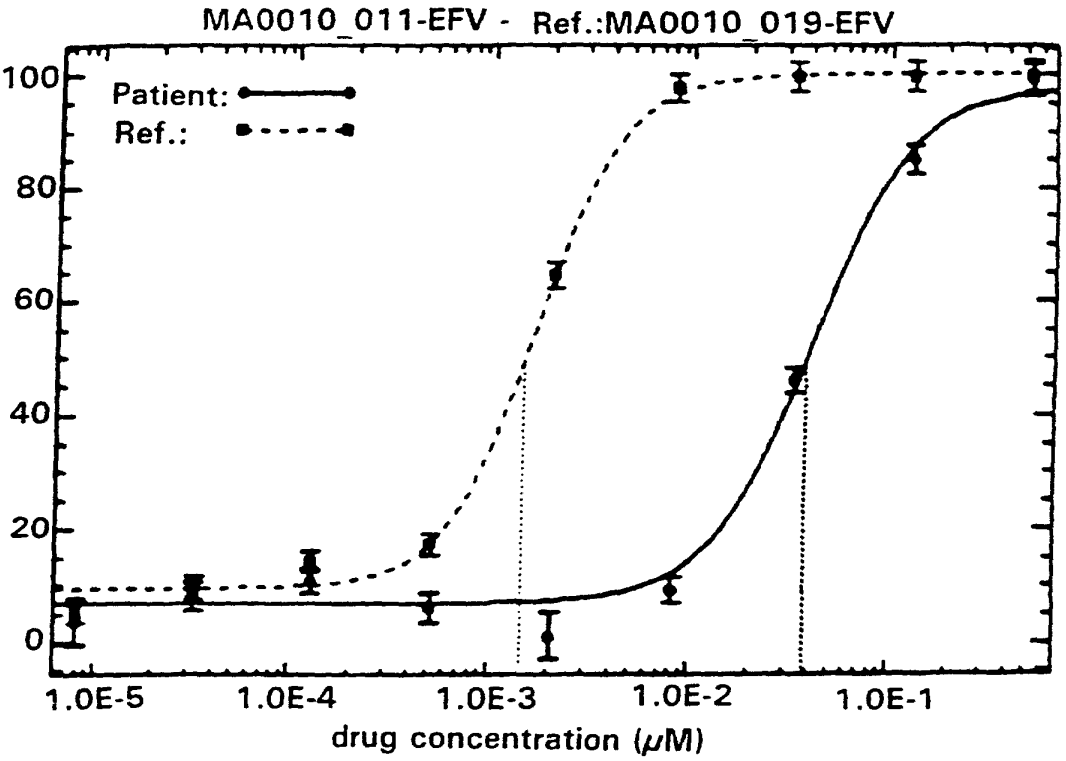
FIG. 3A

NRTI - AZT



AZT-Control	$IC_{50} = 0.032$
AZT-Patient	$IC_{50} = 0.170$ (5.2-fold)

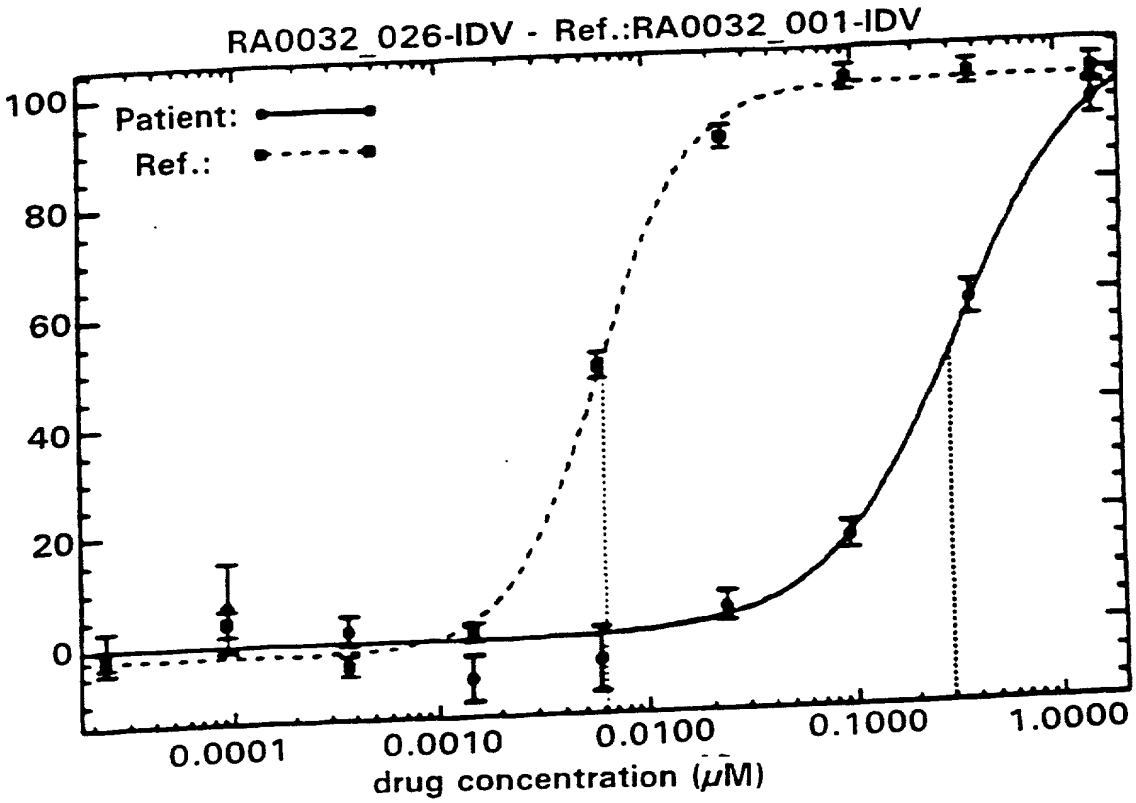
FIG. 3B **NNRTI - Efavirenz**



EFV-Control	IC_{50} = 0.0015
EFV-Patient	IC_{50} = 0.0380 (25.6-fold)

FIG. 3C

PRI - Indinavir



IDV-Control	IC_{50} = 0.0062
IDV-Patient	IC_{50} = 0.2935 (47.4-fold)

FIG. 4A **SQV**

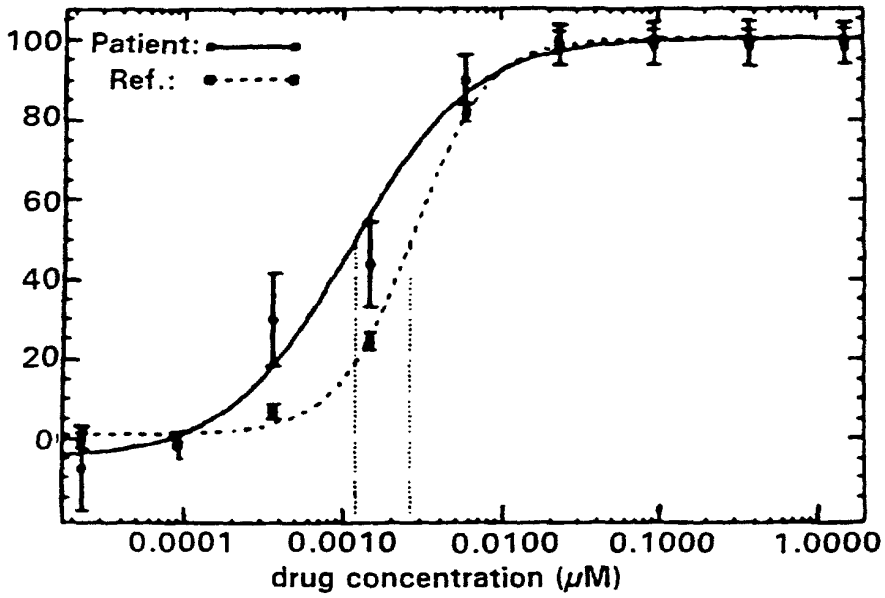


FIG. 4B **IDV**

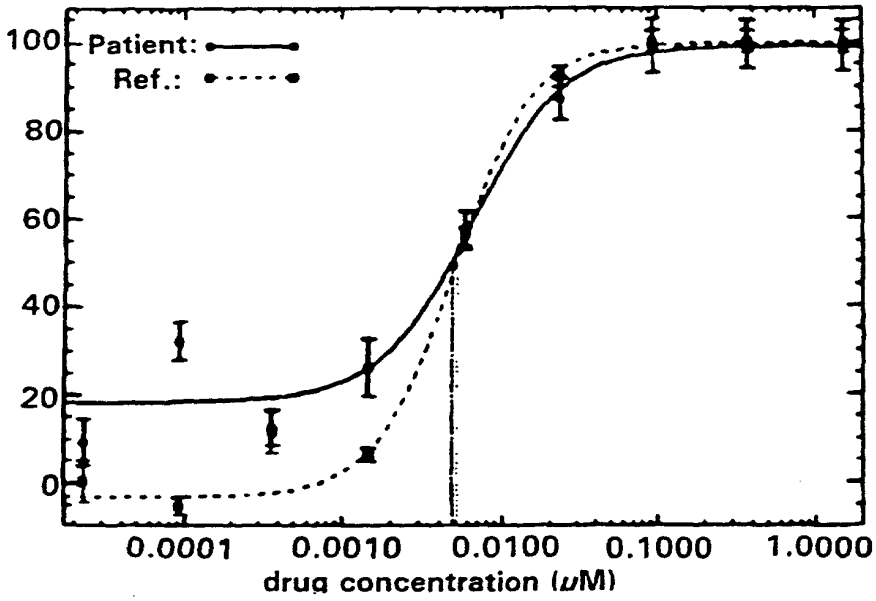


FIG. 4C **RTV**

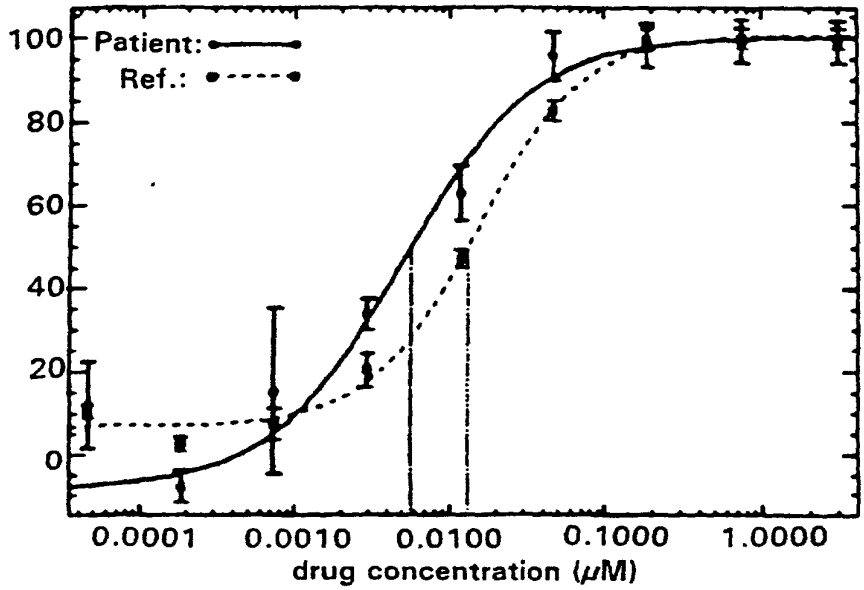


FIG. 4D **NFV**

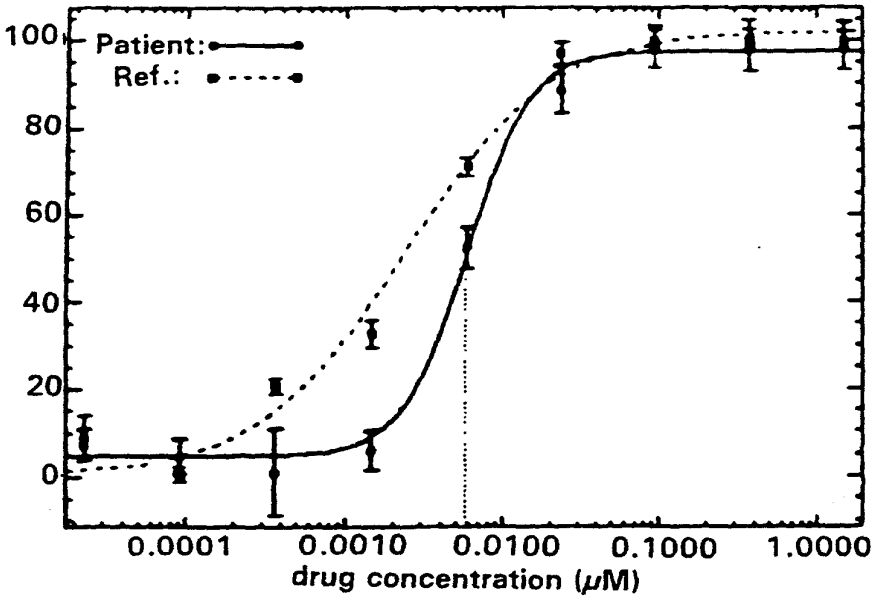


FIG. 4E **AMP**

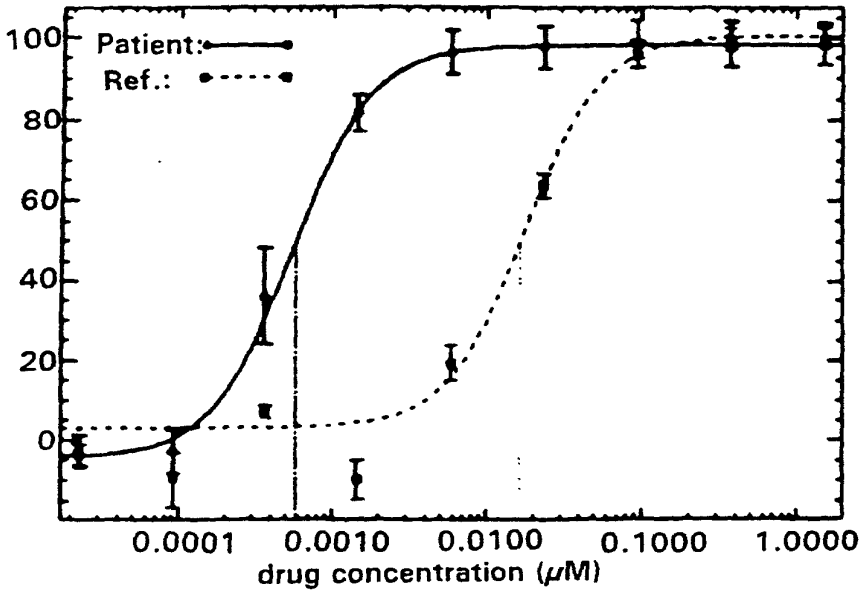

100
80
60
40
20
0
Patient:
Ref.:
0.0001
0.0010
0.0100
0.1000
1.0000
drug concentration (μM)

FIG. 5A **SQV**

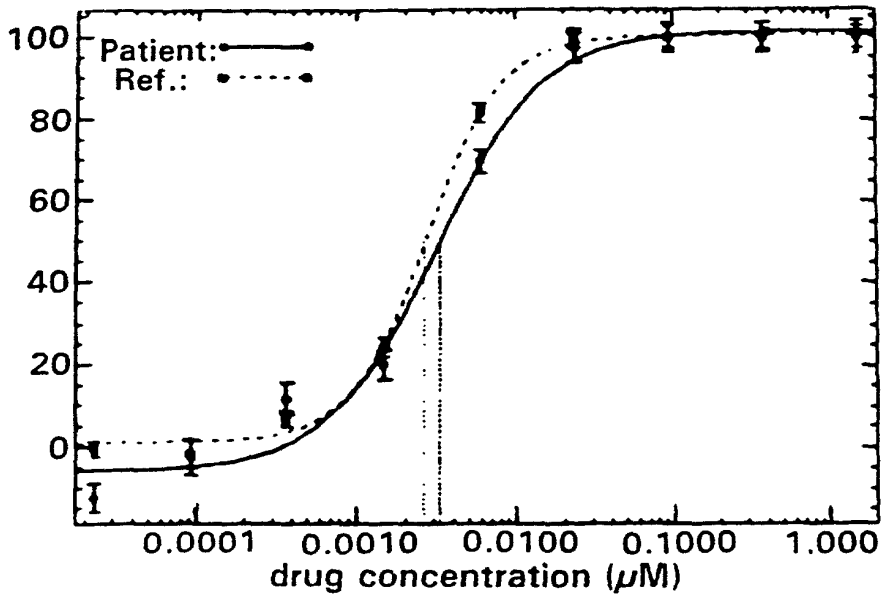


FIG. 5B **IDV**

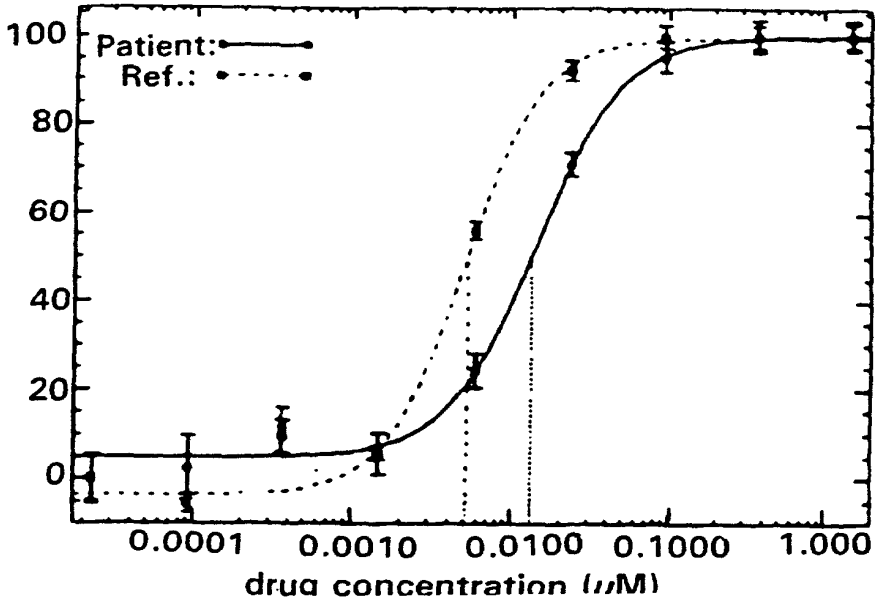


FIG. 5C **RTV**

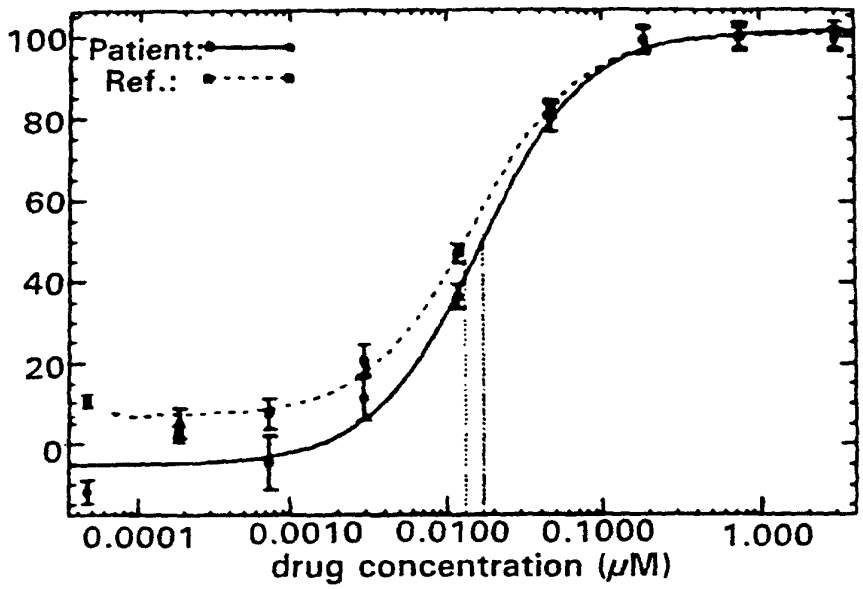


FIG. 5D **NFV**

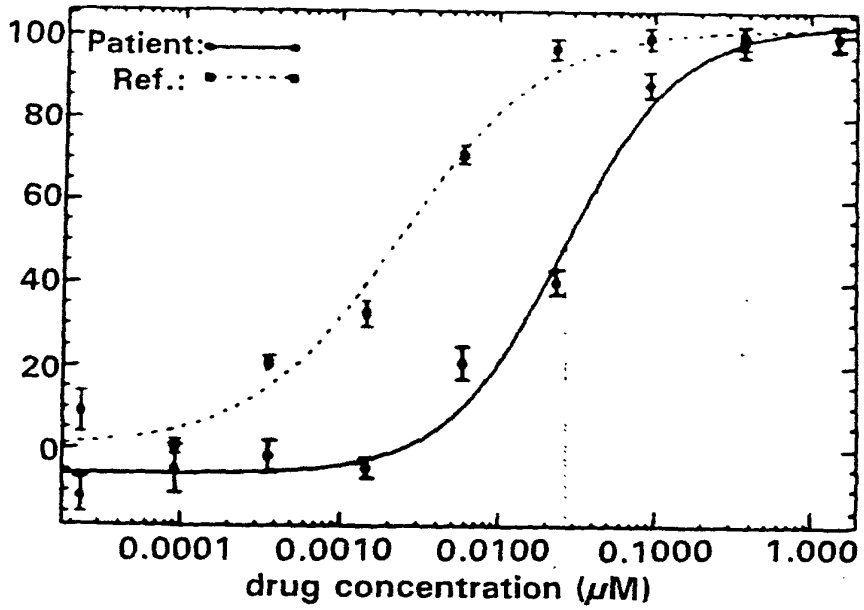


FIG. 5E **AMP**

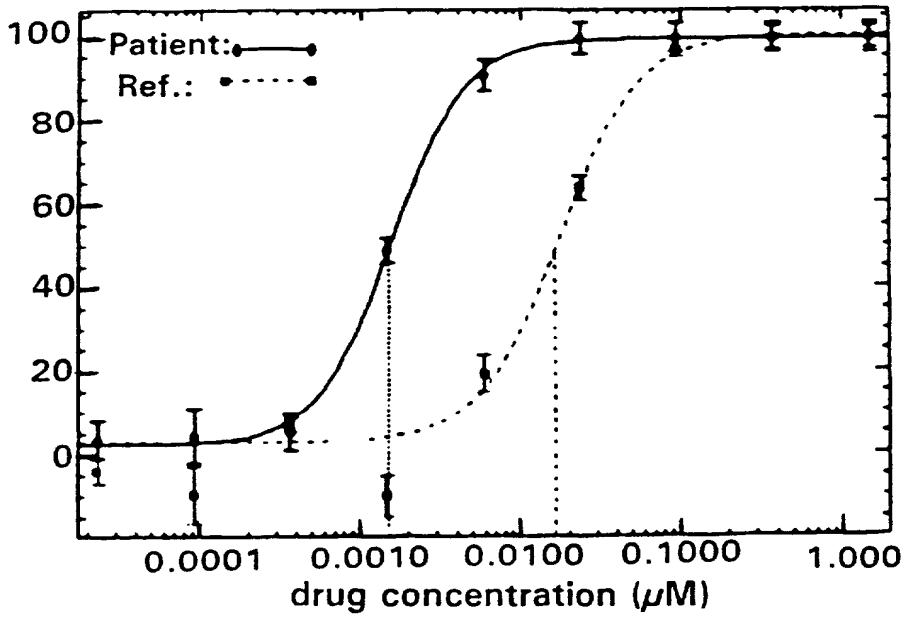

100
80
60
40
20
0
Patient:
Ref.:
0.0001
0.0010
0.0100
0.1000
1.000
drug concentration (μM)

Figure A: Fitness Assay

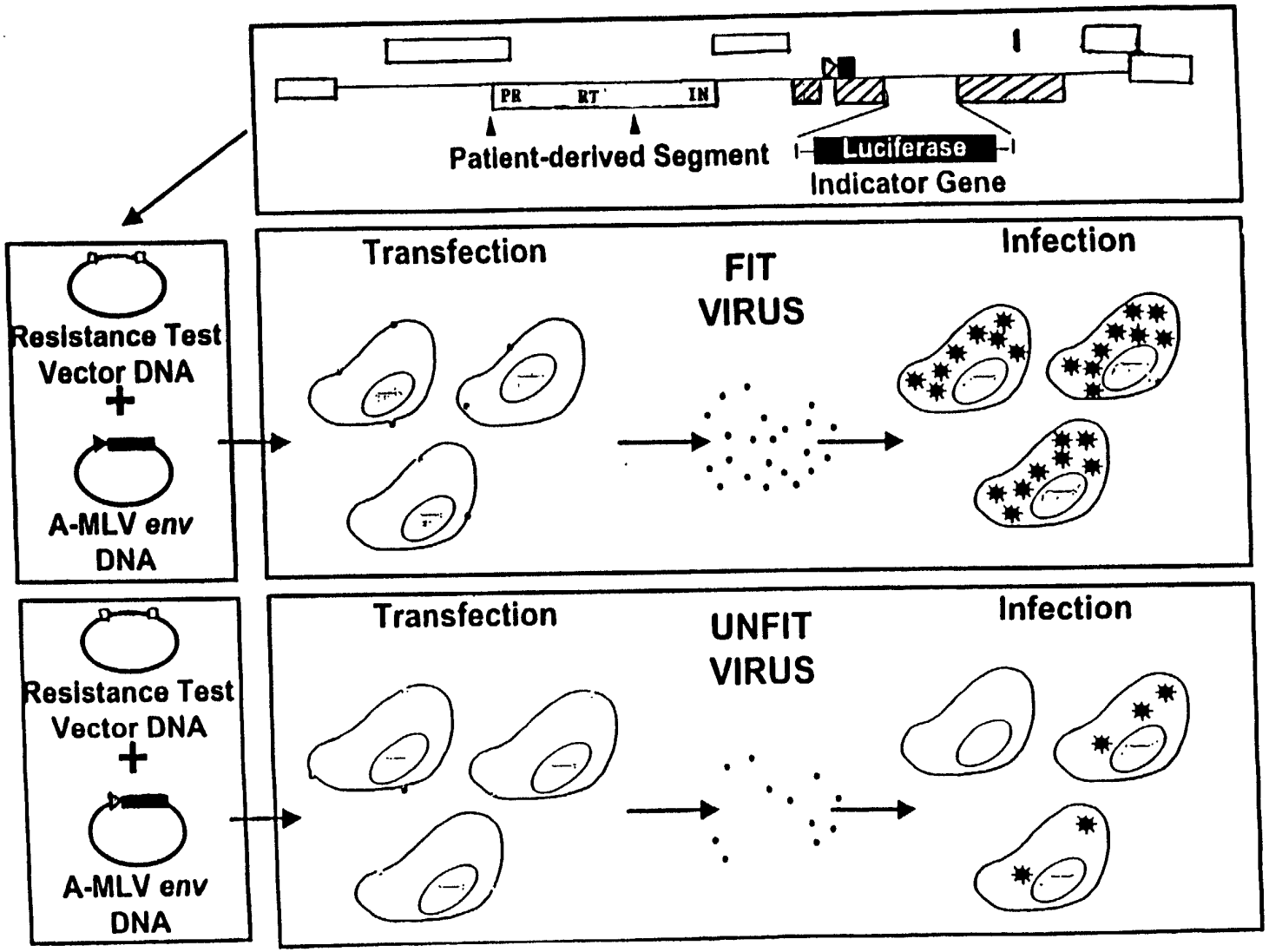
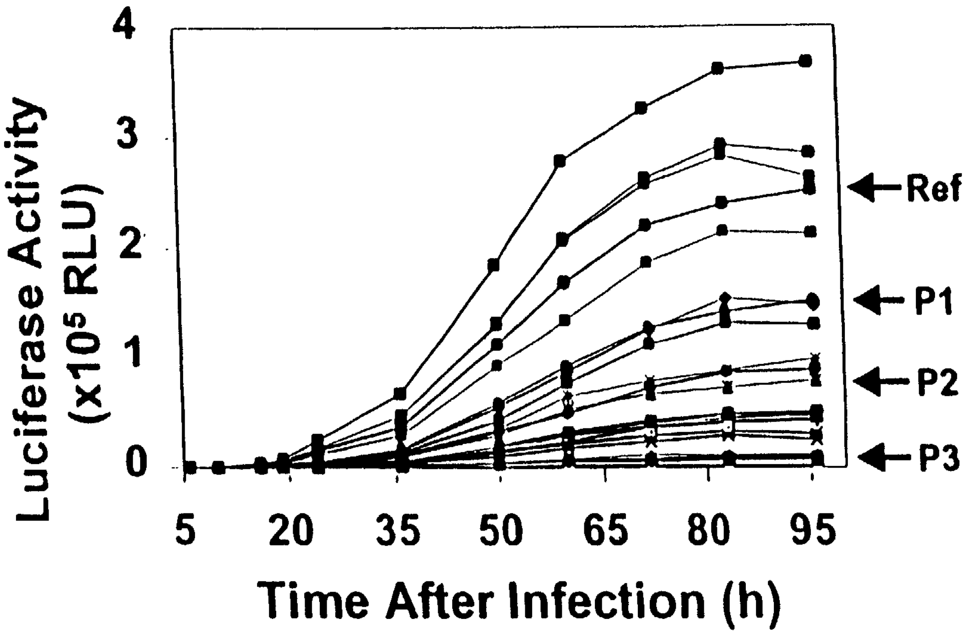


Figure B: Luciferase Activity in Infected Cells



Fold Resistance

		P 1	P 2	P 3
NRTI	AZT	27	17	6
	3TC	>100	3	>100
NNRTI	NVP	40	0.3	0.3
	SQV	17	68	4
	IDV	30	47	39
PRI	RTV	11	62	63
	NFV	57	55	28
	AMP	4	18	3

Figure C: Replication Fitness, PR Processing, and RT Activity

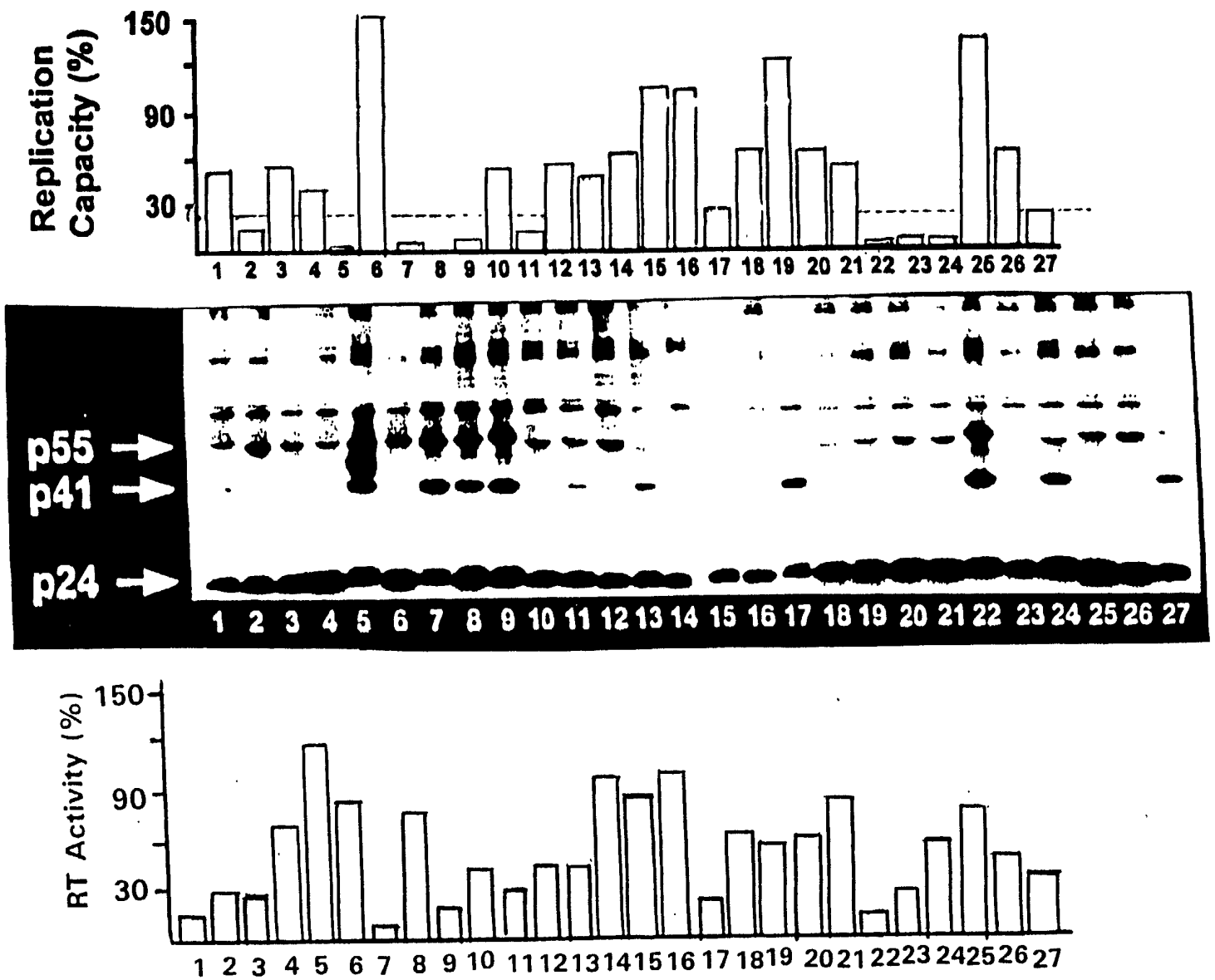
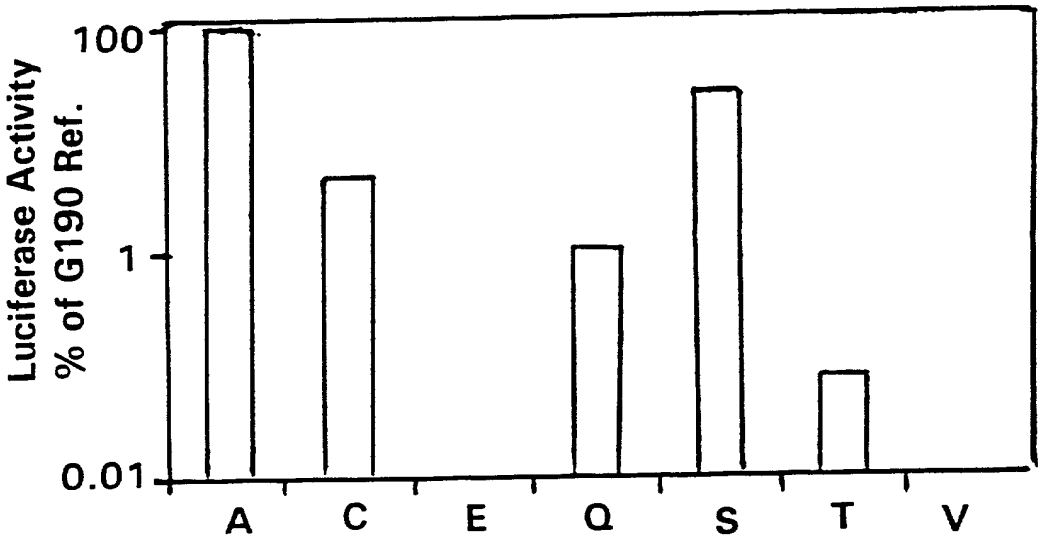
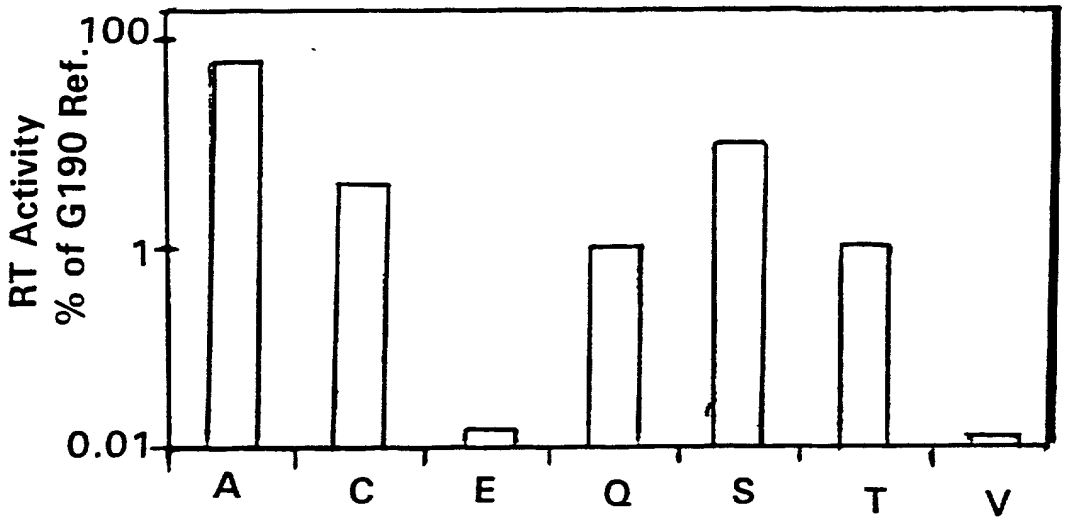


Figure D: Site Directed RT Mutants (G190 Series)

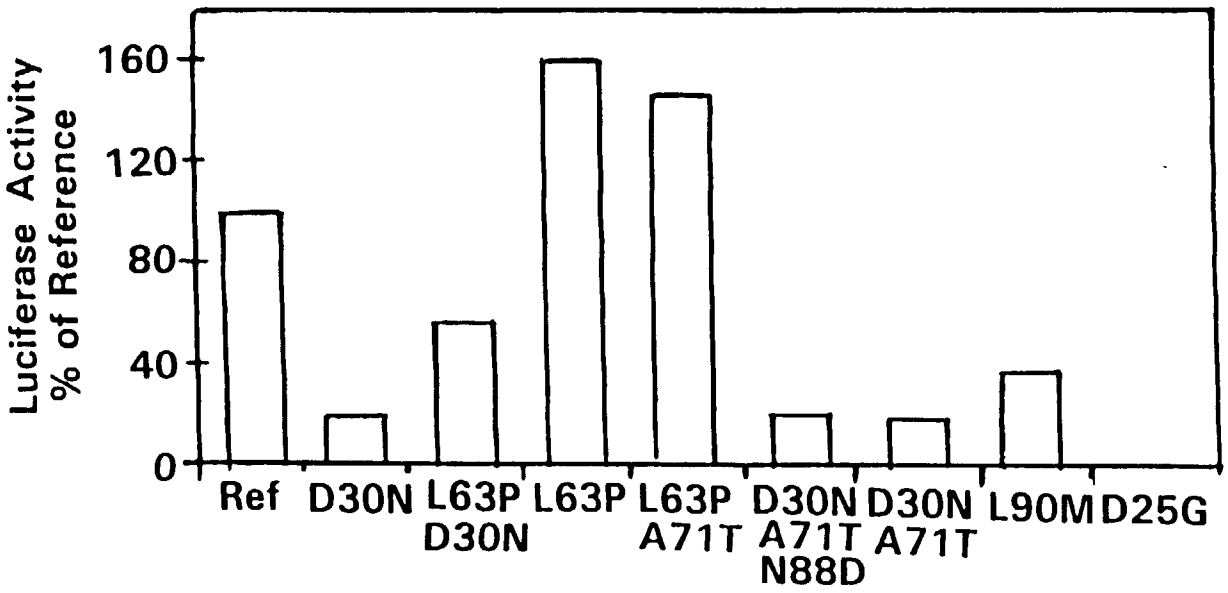




G190 Mutants

A = Ala	C = Cys
E = Glu	Q = Gln
S = Ser	T = Thr

Figure E: Site Directed PR Mutants



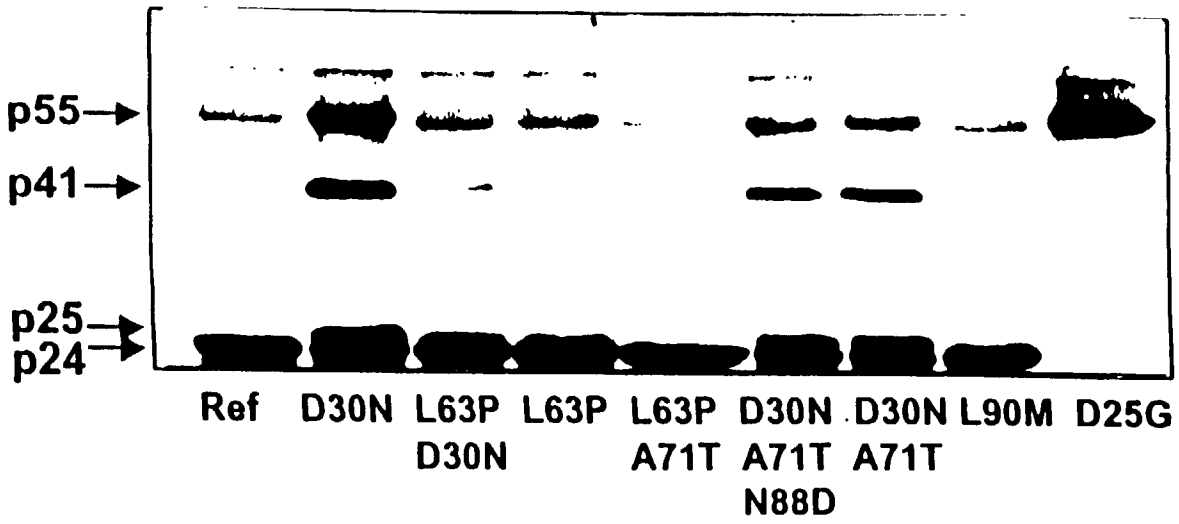


Figure F: Phenotypic Drug Susceptibility, Replication Fitness and PR/RT Function

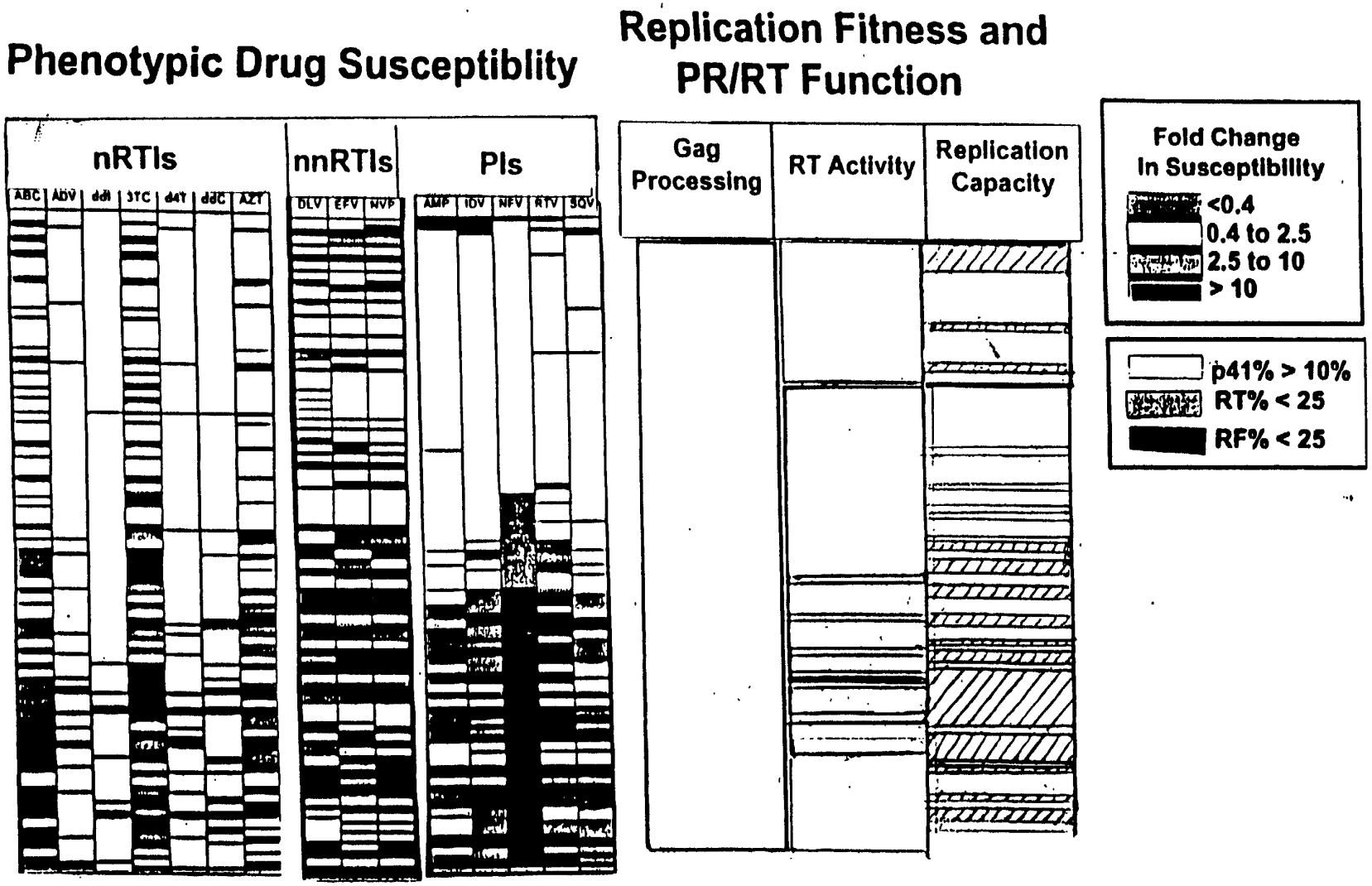


Figure G: Relation of PI Resistance to Replication Capacity

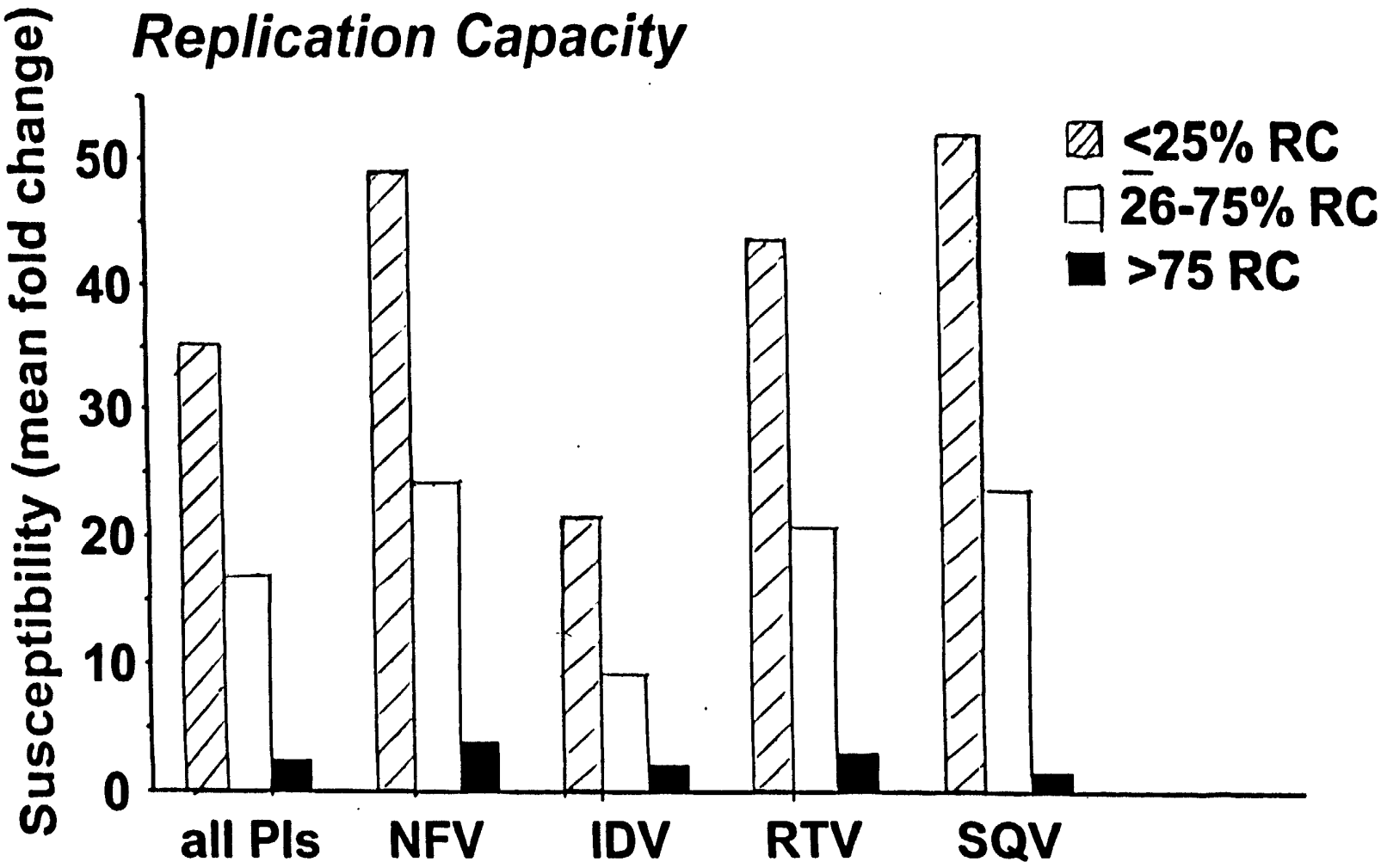


Figure H: Relation of NRTI and NNRTI Resistance to Replication Capacity

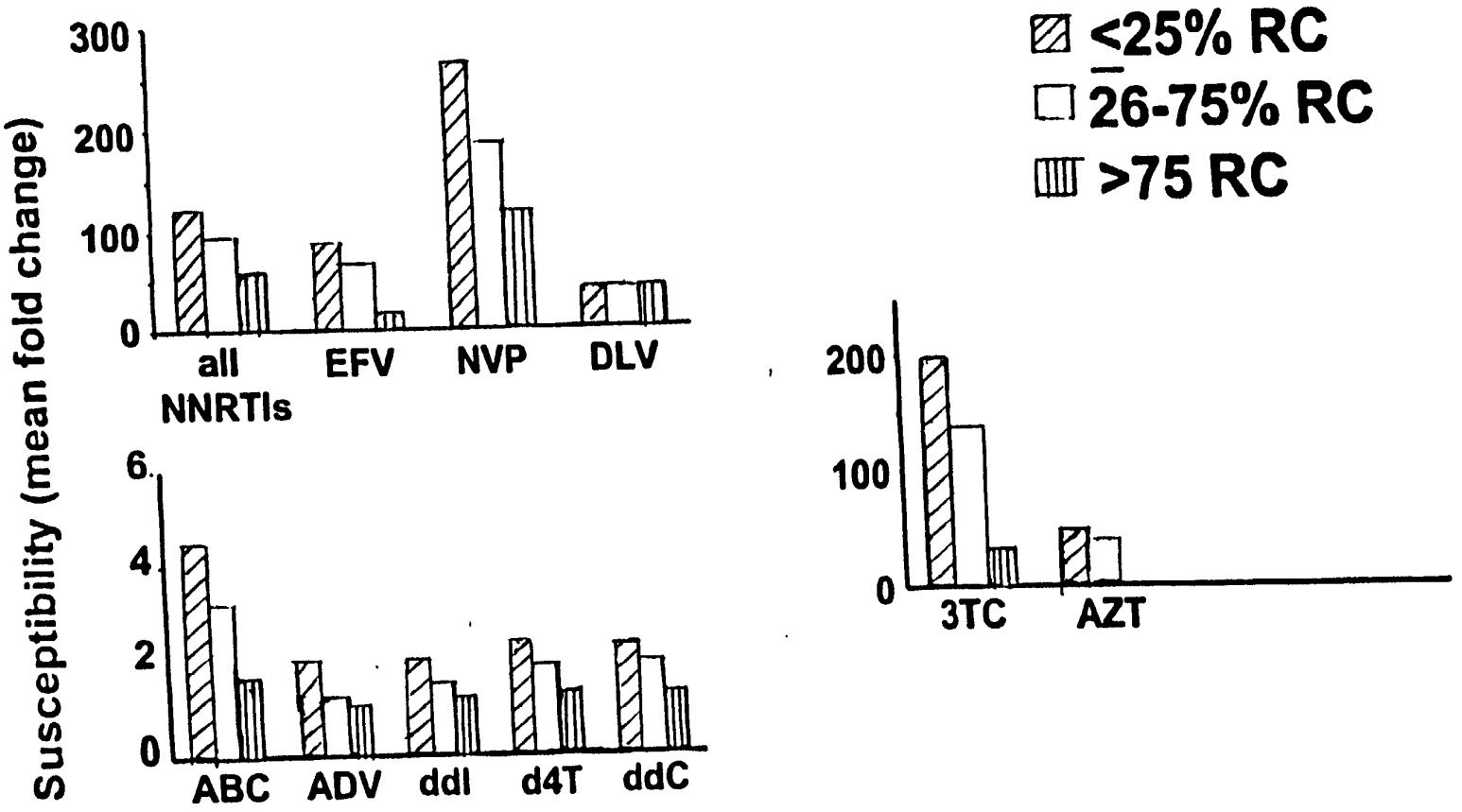


Figure I: Low Replication Capacity is Associated with High Numbers of Mutations in Protease and L90M

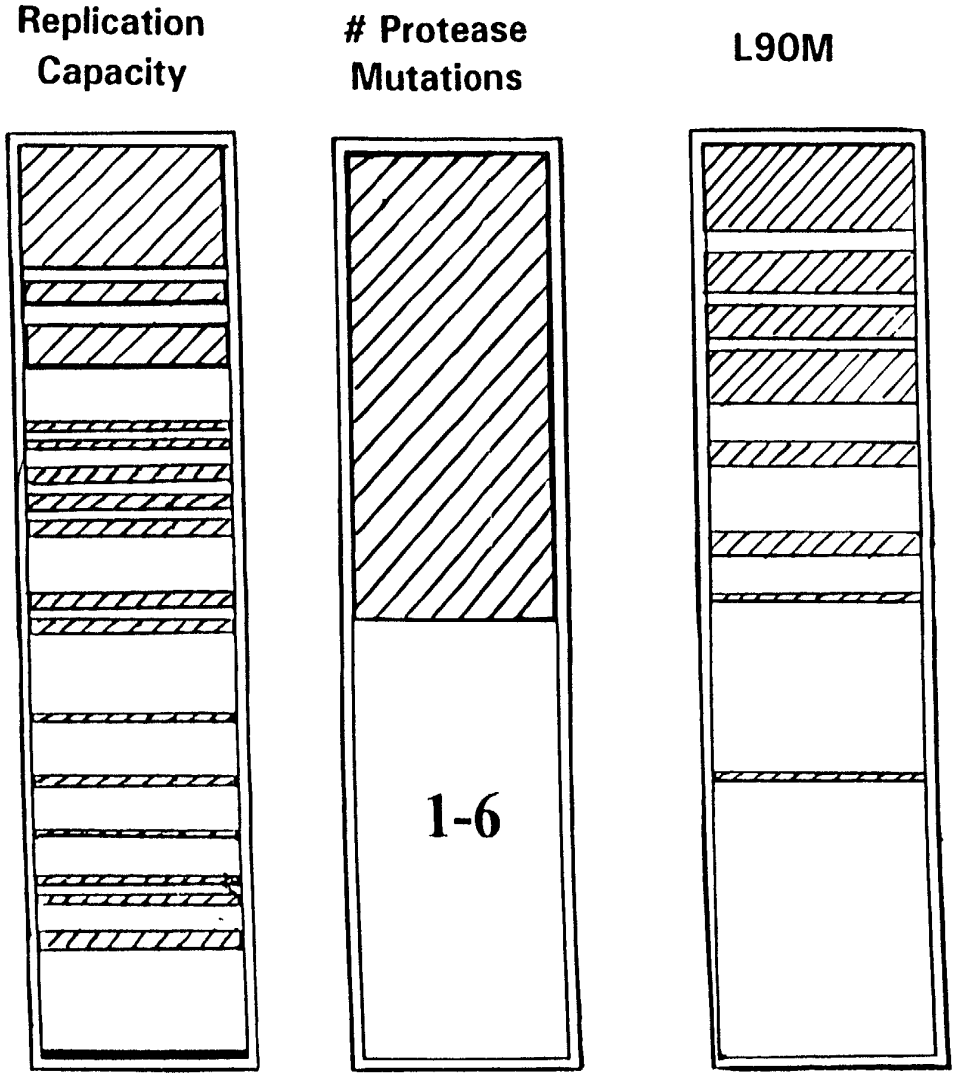


Figure J: Low Replication Capacity is Associated With Specific Protease Mutations

- **D30N**
- **L90M PLUS mutations at 73, 20, 46, or 88**

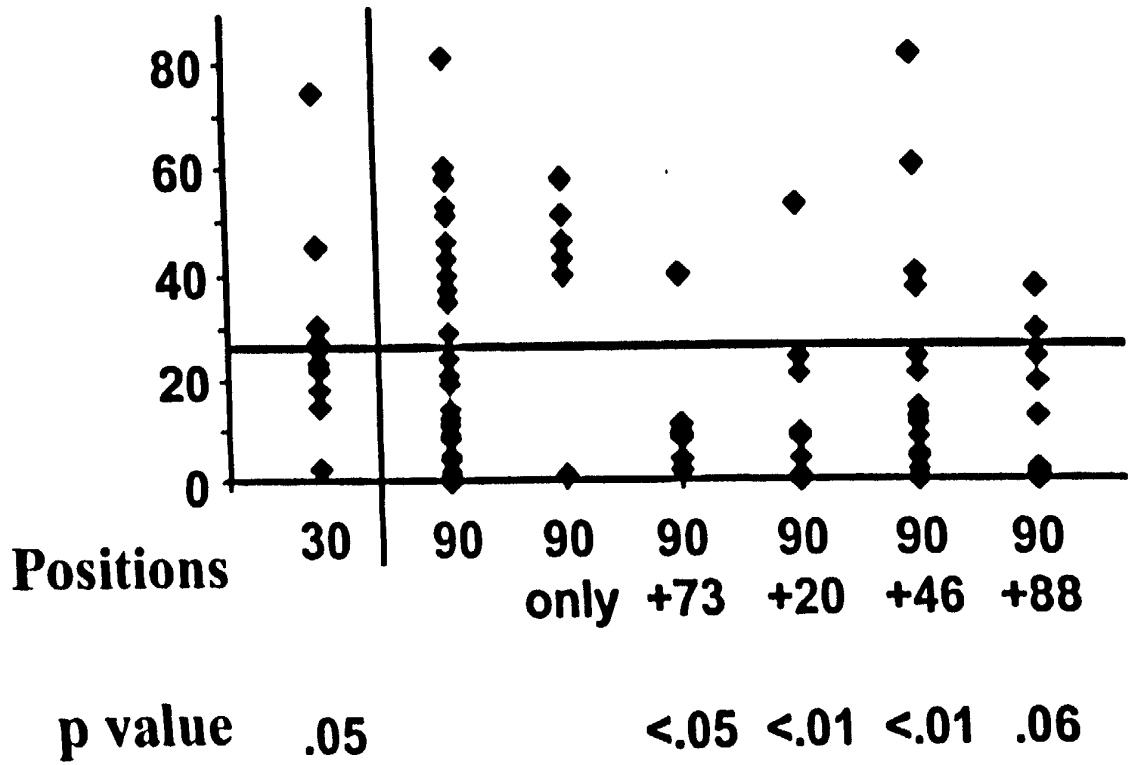


Figure K: Relation of NFV Phenotypic Drug Susceptibility, gag Processing and Replication Fitness

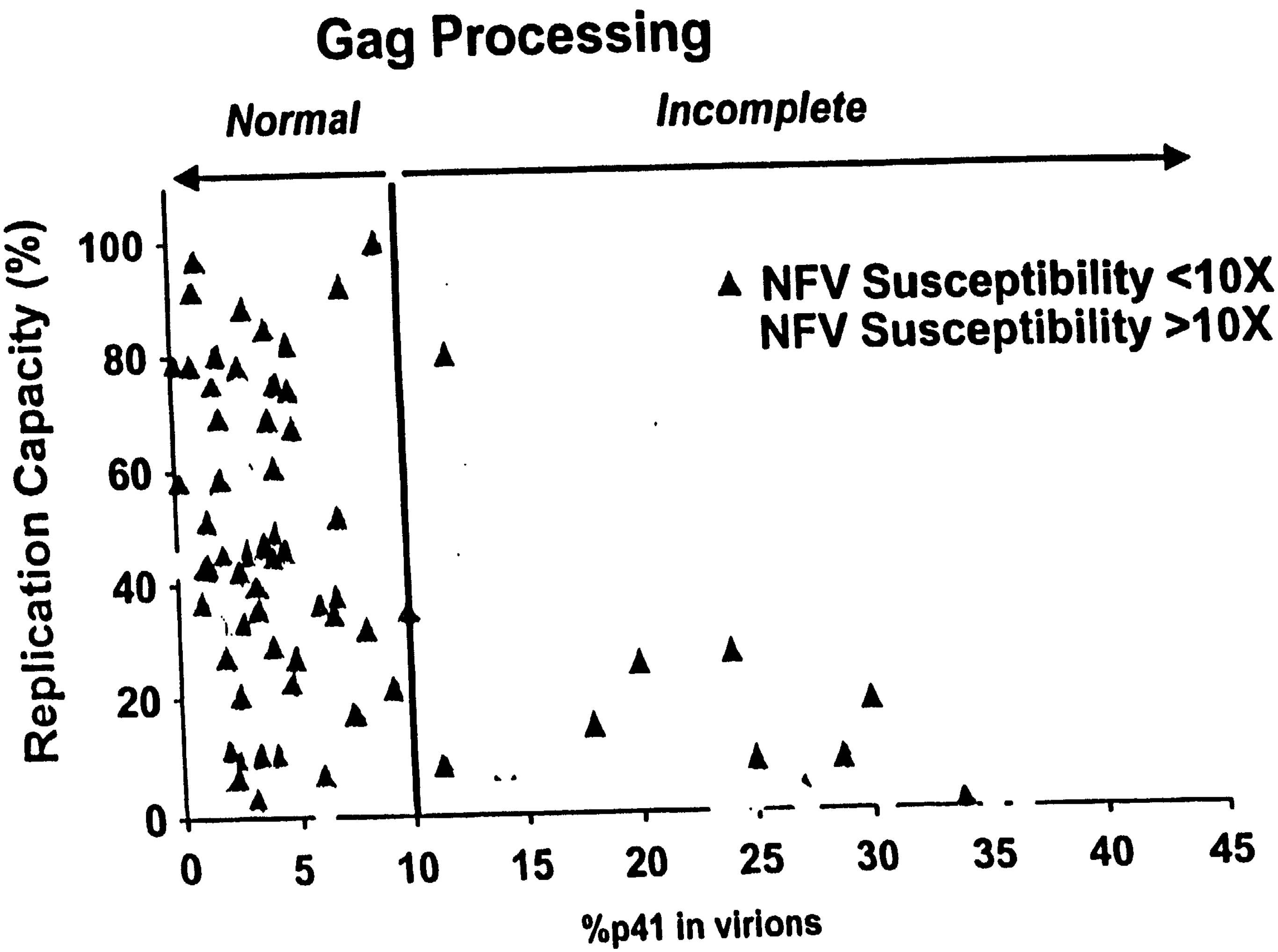
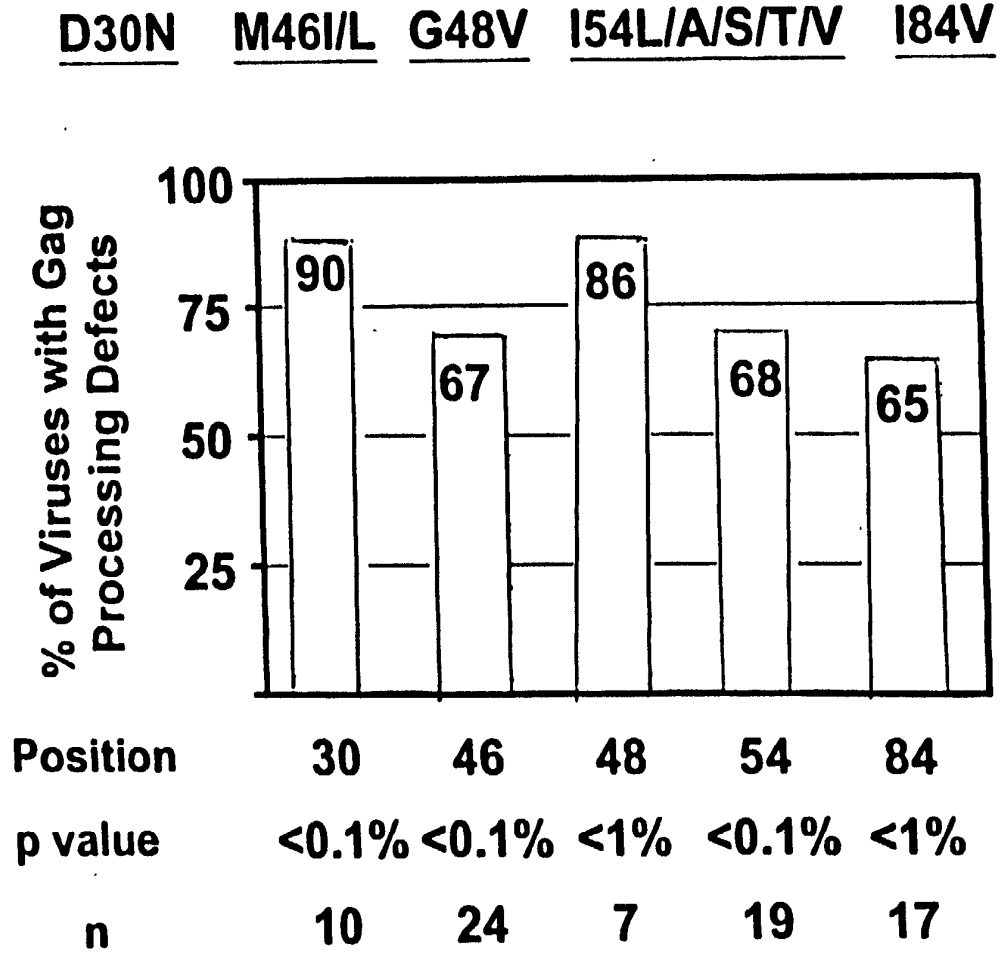


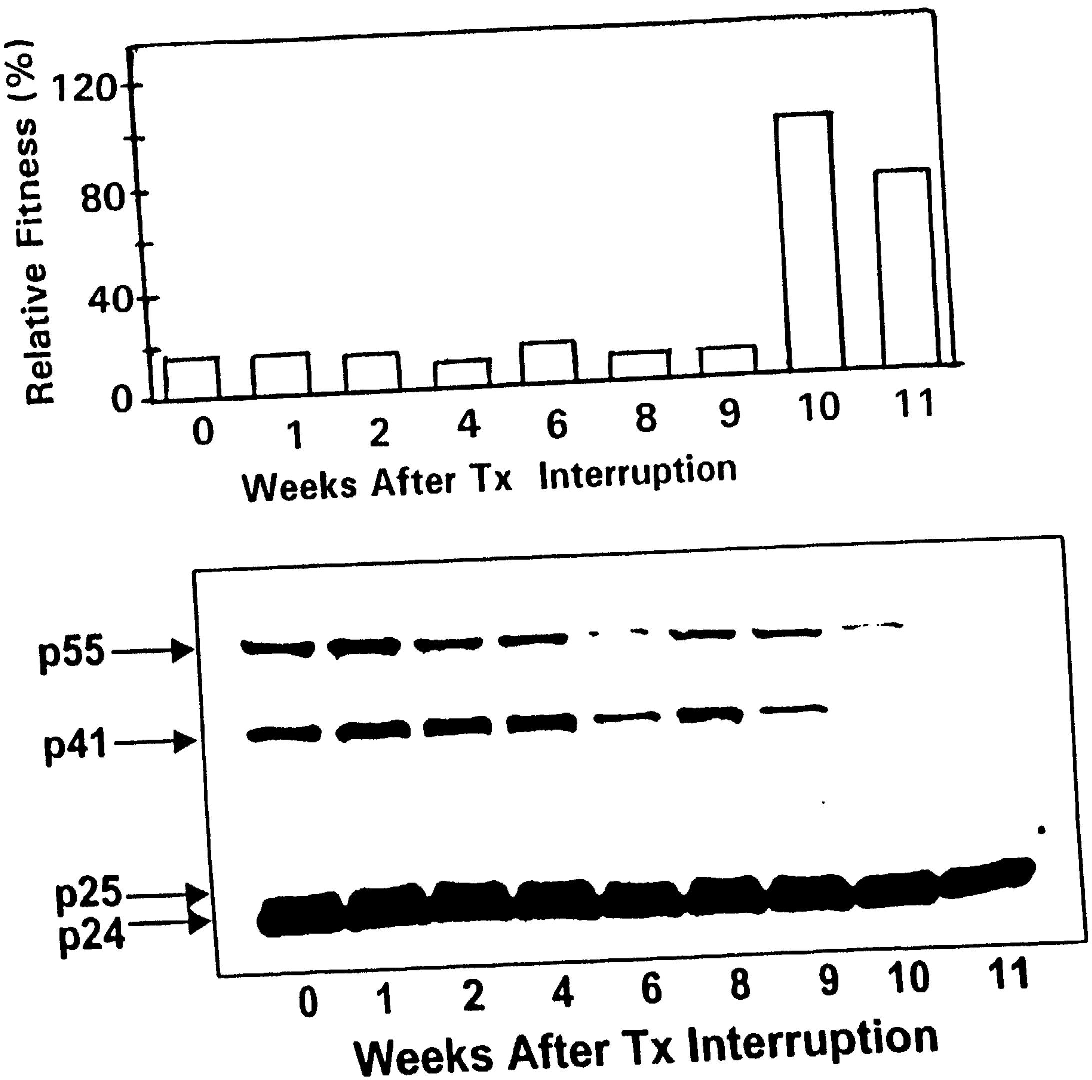
Figure L: Mutations in PR Associated with Gag Processing Defects



	NRTI				NNRTI			PI				
WEEK	AZT	3TC	D4T	ABC	NVP	DLV	EFV	SQV	IDV	RTV	NFV	AMP
day 0	3.7	>100	2.8	19	>300	88	115	85	72	73	74	16
1	4.5	>100	3.3	20	>300	78	134	95	74	59	80	21
2	5.8	>100	3.2	14	>300	75	142	89	77	49	59	19
3	6.5	>100	2.7	15	>300	96	183	59	75	52	51	15
4	6.3	>100	3.1	15	>300	94	174	59	68	50	49	15
5	6.4	>100	3.0	17	>300	76	119	59	60	54	36	10
6	5.0	>100	2.8	19	>300	93	168	89	39	80	40	18
7	9.1	>100	4.1	12	>300	89	154	85	78	53	53	19
9	2.8	8.1	1.9	5.0	22	15	10	1.8	3.5	4.7	4.0	2.0
10	1.5	1.7	1.1	1.3	1.7	2.0	1.6	0.9	1.6	1.9	1.8	1.6
11	0.9	1.2	1.0	1.2	0.8	1.1	0.9	1.0	1.1	1.1	1.1	1.0
12	0.8	1.3	0.8	1.2	0.5	1.0	0.8	0.8	0.8	0.9	1.1	0.8
23	0.7	1.1	1.0	0.6	0.8	1.1	0.8	0.8	0.8	1.0	0.9	0.6

Figure M: Patient Virus Reversion to Drug Susceptibility after Treatment Interruption

Figure N: Patient Virus Reversion to Normal Replication Fitness after Treatment Interruption



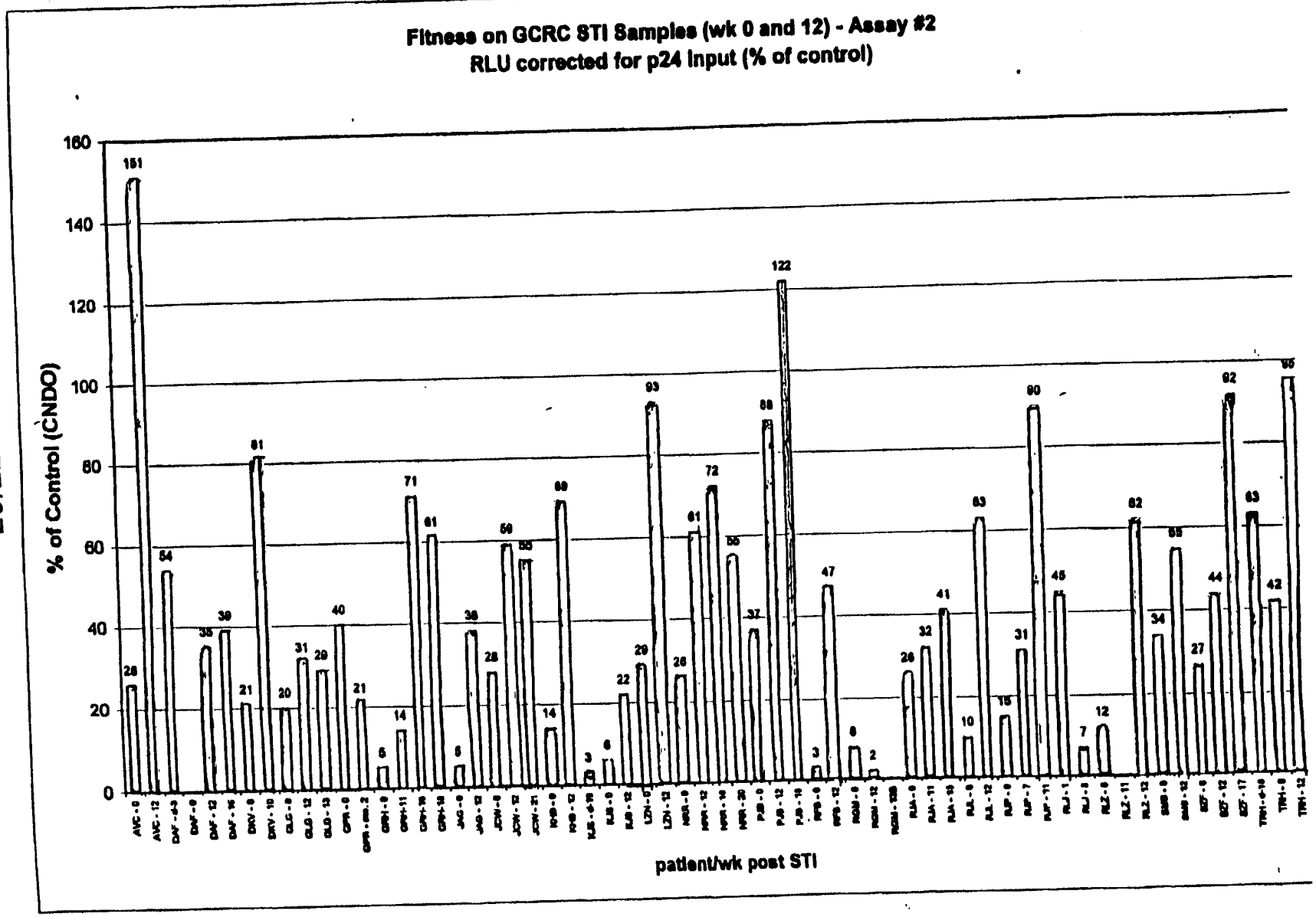

Fitness on GCRC STI Samples (wk 0 and 12) - Assay #2
RLU corrected for p24 Input (% of control)
% of Control (CNDO)
patient/wk post STI
0
20
40
60
80
100
120
140
160

Figure P:

To Measure Replication Capacity of Patient-Derived Recombinant Viruses

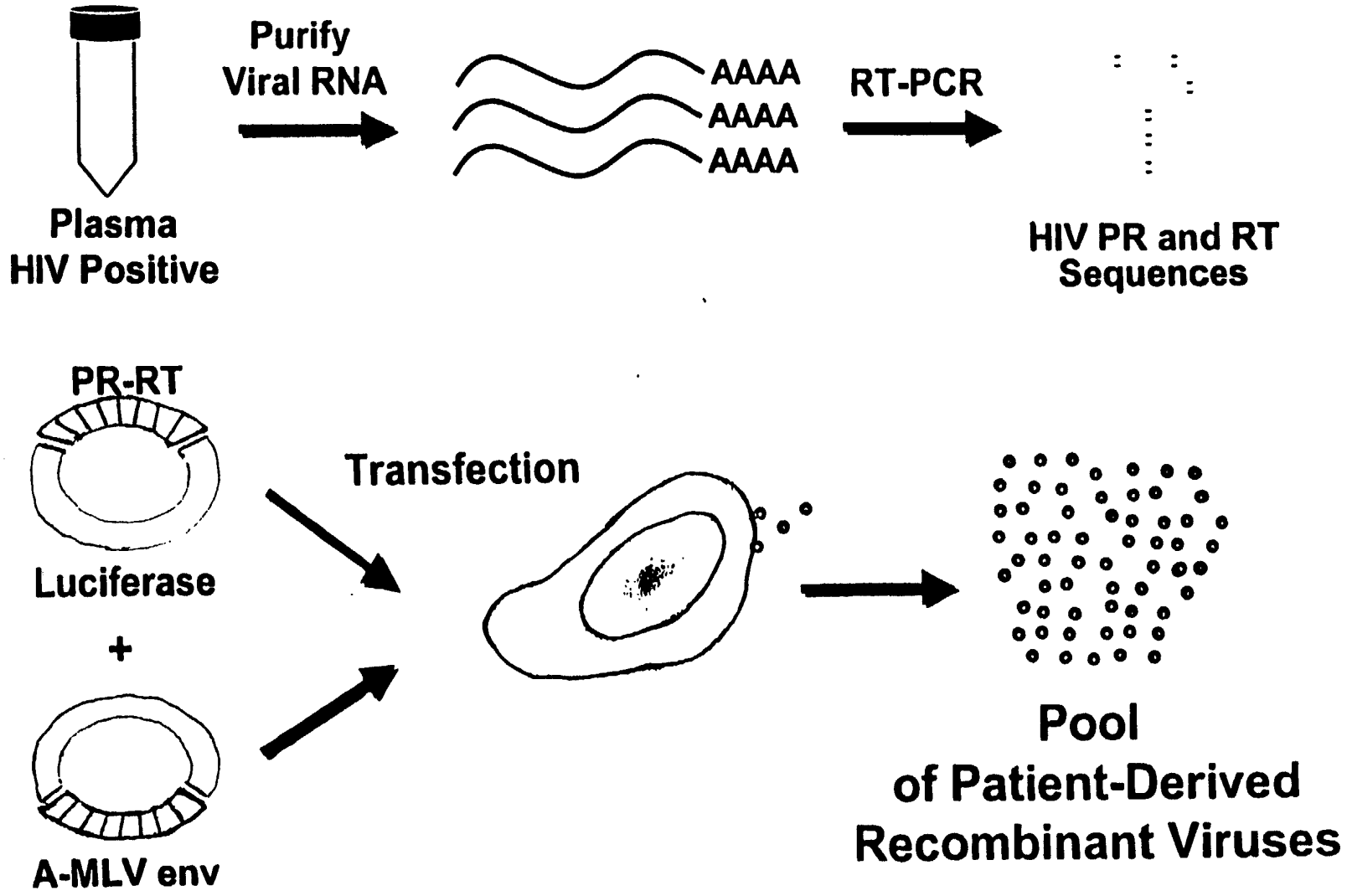


Figure Q:

To Measure Replication Capacity of Patient-Derived Recombinant Viruses

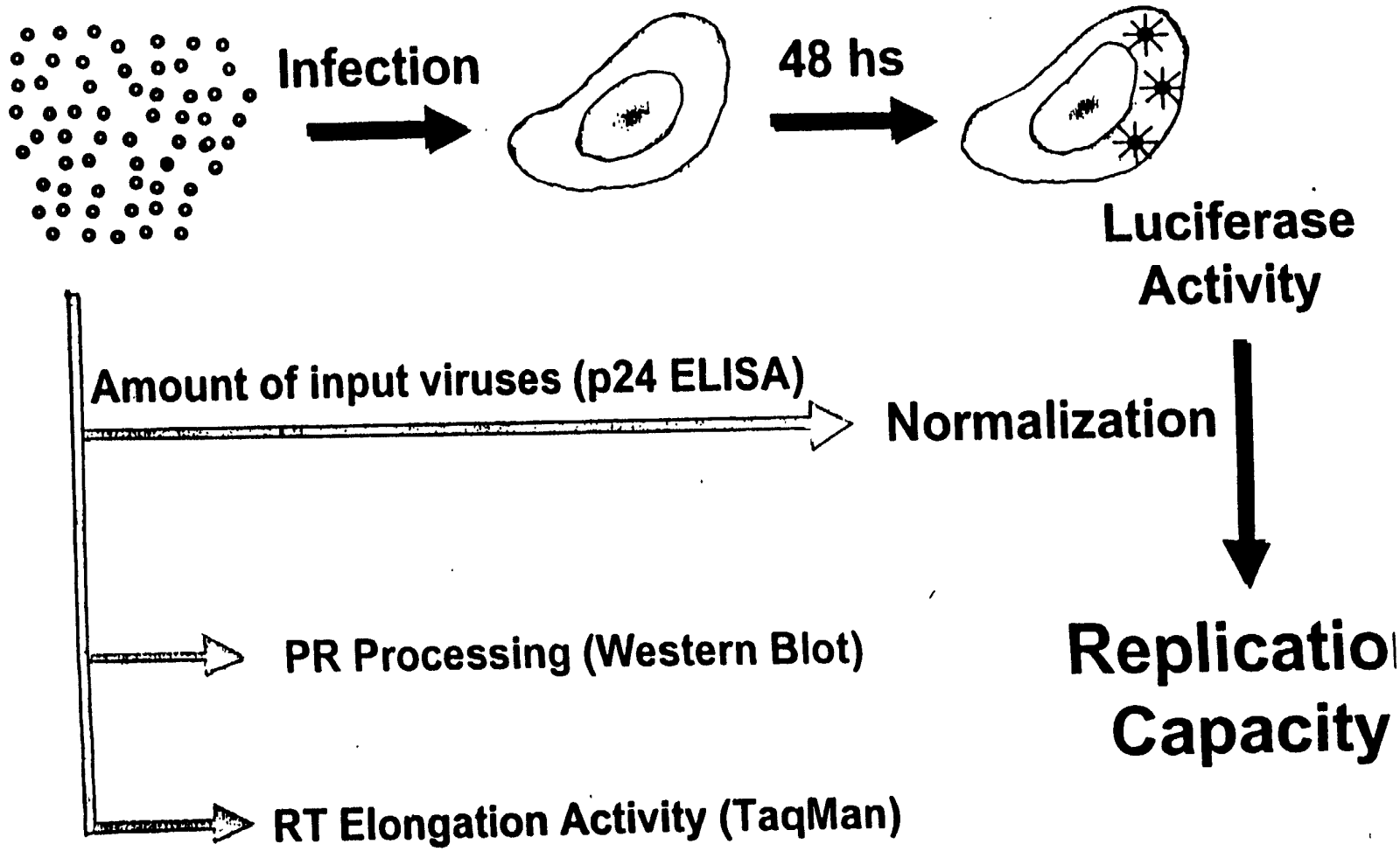
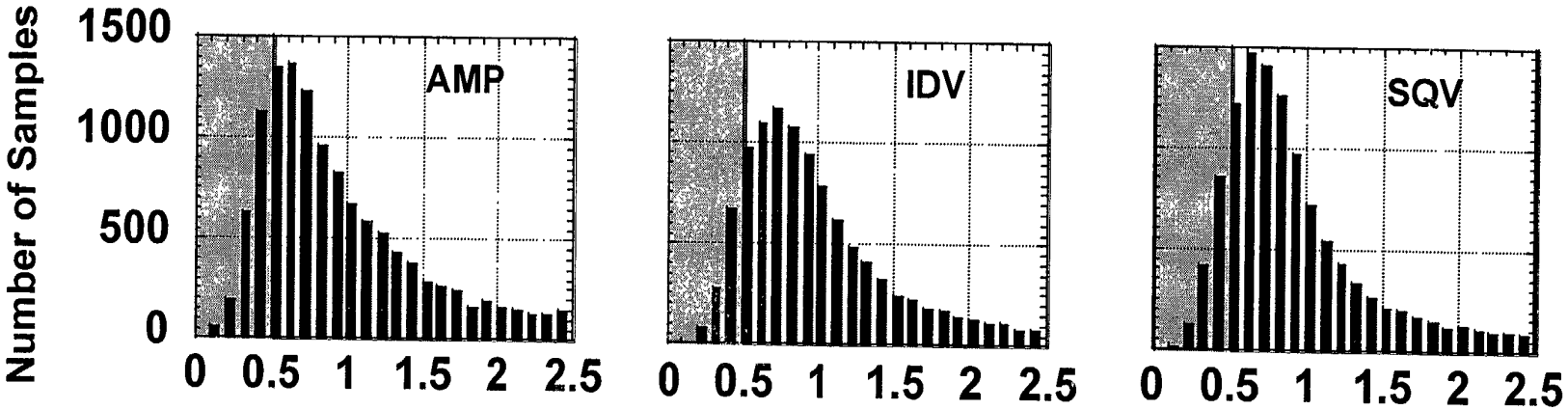


Figure 6

Distribution of Fold Change in IC50s to Protease Inhibitors of Susceptible Viruses

in a Database of 17000 Samples



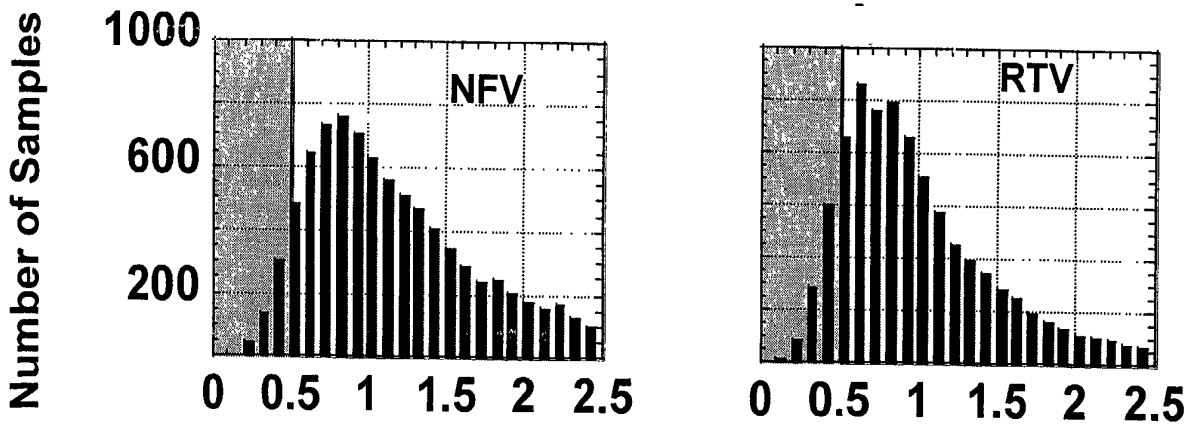


Figure 7

Fold Change Susceptibility

20 Randomly Selected Patient Derived Viruses with HS to PIs

	RT Inhibitors									PR Inhibitors				
Sample	ABC	ddI	3TC	d4T	ddC	ZDV	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
1	3.2	2.2	>300	0.9	1.7	1.2	0.9	41.9	>700	0.4	0.6	1.1	0.4	0.3
2	1.0	1.0	1.3	1.1	1.1	0.7	1.2	0.8	0.8	0.6	0.3	0.7	0.2	0.3
3	3.1	1.7	>300	0.9	nd	0.7	nd	1.1	0.8	0.2	0.4	0.6	0.4	0.3
4	3.3	1.9	>300	1.0	2.4	1.2	62.9	101	429	0.2	0.4	0.6	0.4	0.2
5	3.6	2.2	5.0	1.7	3.2	0.6	>190	>320	>700	0.2	0.4	0.6	0.5	0.3
6	7.5	1.4	>300	1.4	2.1	22.9	12.8	135	>700	0.5	0.5	0.6	0.4	0.4
7	8.5	1.9	>300	3.7	3.4	73.9	30.6	>320	>700	0.3	0.4	0.6	0.3	0.4
8	2.7	1.6	>300	1.0	1.8	1.1	>190	89.3	>700	0.4	0.4	0.5	0.6	0.4
9	2.0	1.1	>300	0.7	1.3	0.8	8.0	72.1	165	0.3	0.4	0.5	0.3	0.5
10	2.4	1.7	>300	1.2	1.9	0.6	71.5	38.7	109	0.4	0.4	0.4	0.4	0.4
11	2.8	1.5	>300	0.7	1.7	0.4	30.9	94.9	193	0.4	0.4	0.4	0.5	0.4
12	3.4	1.1	>300	1.0	2.1	0.7	3.2	2.0	2.6	0.3	0.5	0.4	0.5	0.4
13	3.1	2.1	>300	1.1	3.8	0.6	2.4	1.1	1.5	0.3	0.3	0.4	0.3	0.3
14	1.6	1.1	2.0	0.9	1.5	0.9	>190	60.4	>700	0.2	0.3	0.3	0.2	0.2
15	1.2	1.0	1.2	1.1	1.2	1.7	1.2	1.2	1.2	0.2	0.4	0.3	0.4	0.6
16	2.8	1.3	3.5	1.2	1.2	14.3	21.9	12.4	71.8	0.2	0.3	0.2	0.2	0.4
17	3.0	2.0	>300	1.2	1.8	2.0	11.3	22.1	160	0.2	0.2	0.2	0.2	0.2
18	3.9	1.4	>300	1.6	1.5	3.1	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.3
19	3.1	1.1	49.5	1.6	1.5	6.9	13.4	9.9	33.2	0.3	0.2	0.2	0.2	0.2
20	0.9	1.2	1.3	0.9	0.8	1.0	0.8	0.6	0.6	0.3	0.3	0.2	0.3	0.3

0 - 0.4 | 0.4 - 2.5 | 2.5 - 10 | > 10

Figure 8

Replication Capacity of Patient Derived Viruses with HS to PIs

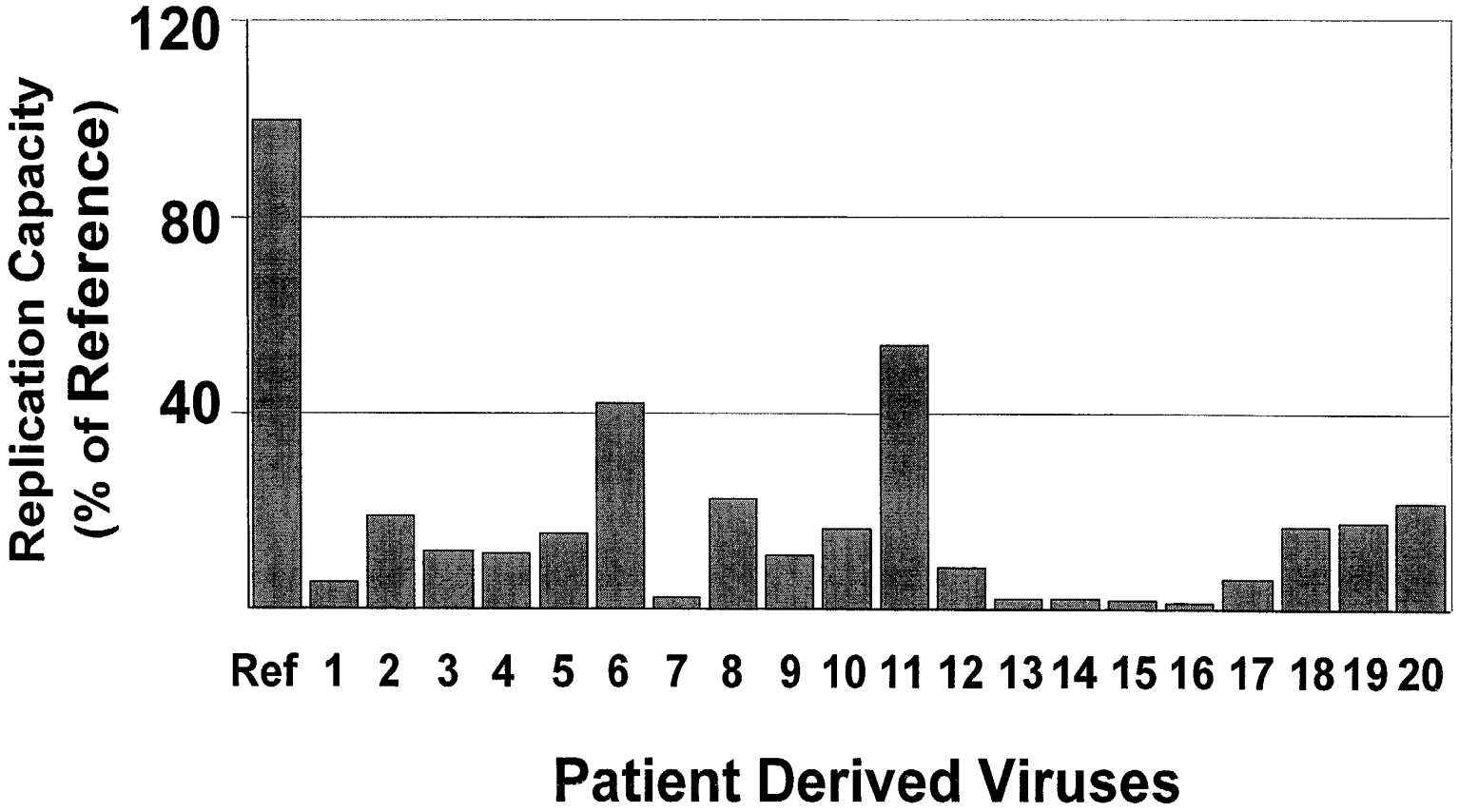


Figure 9

PhenoSense™HIV

Cell based assay to measure phenotypic drug susceptibility employing patient-derived recombinant viruses

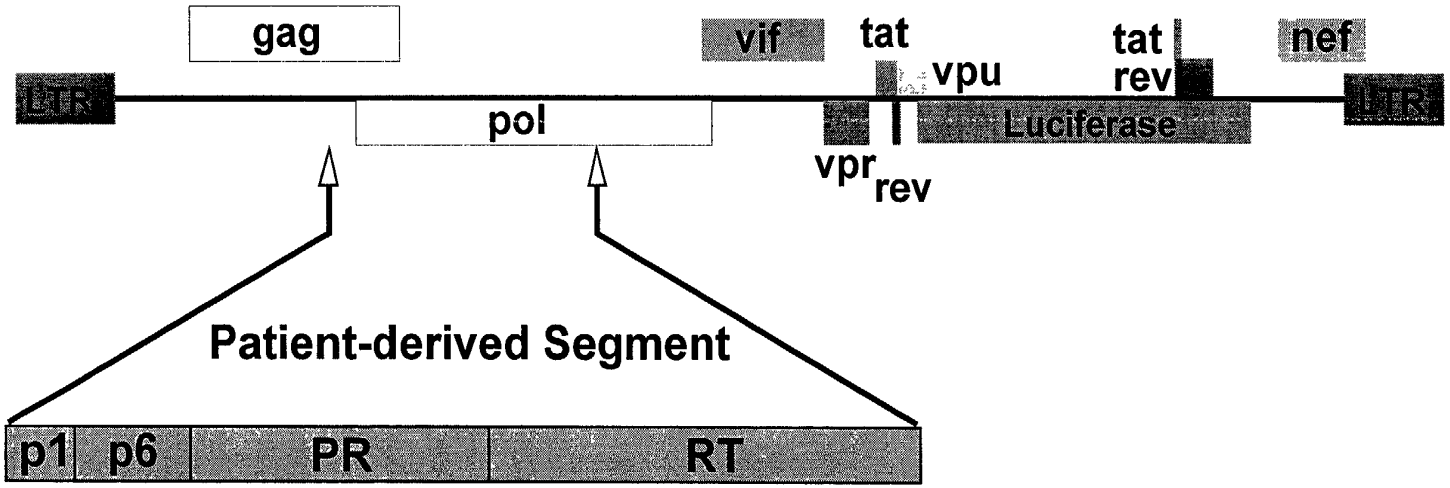


Figure 10

In order to identify mutations responsible for HS and decreased fitness, we used a modified PhenoSense HIV assay employing recombinant viruses carrying different segments from patient isolates:

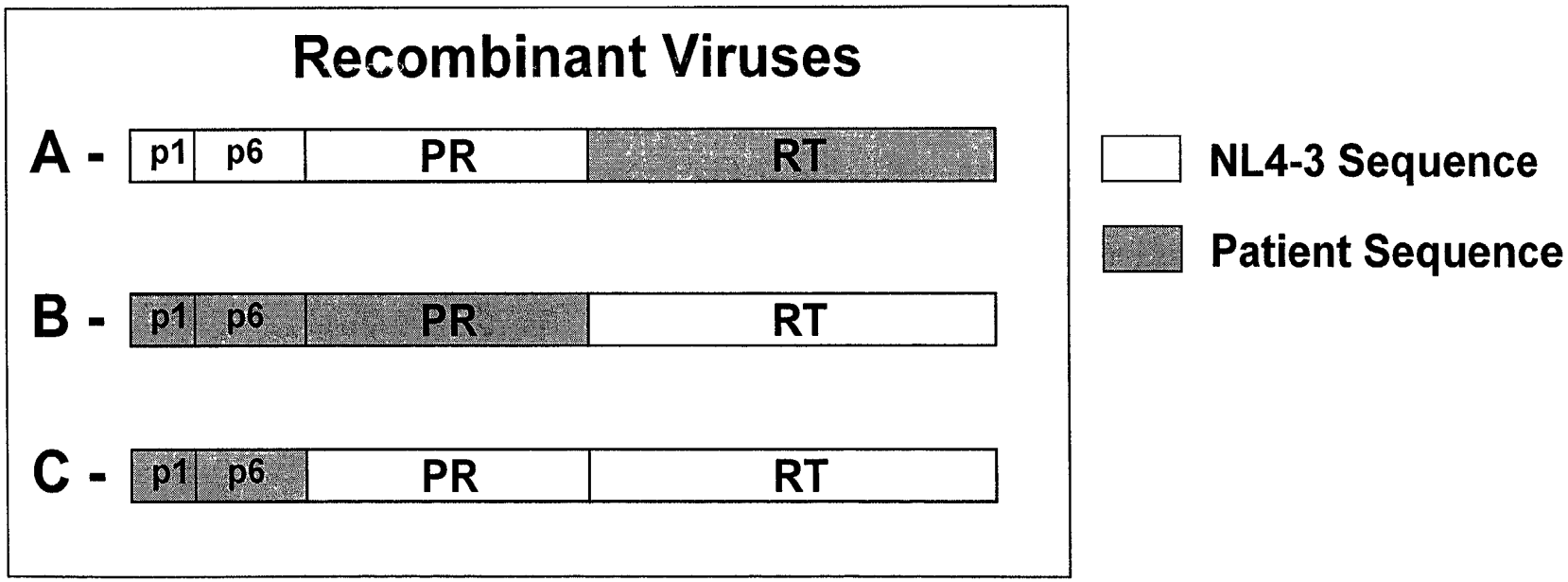


Figure 11

A - [redacted]

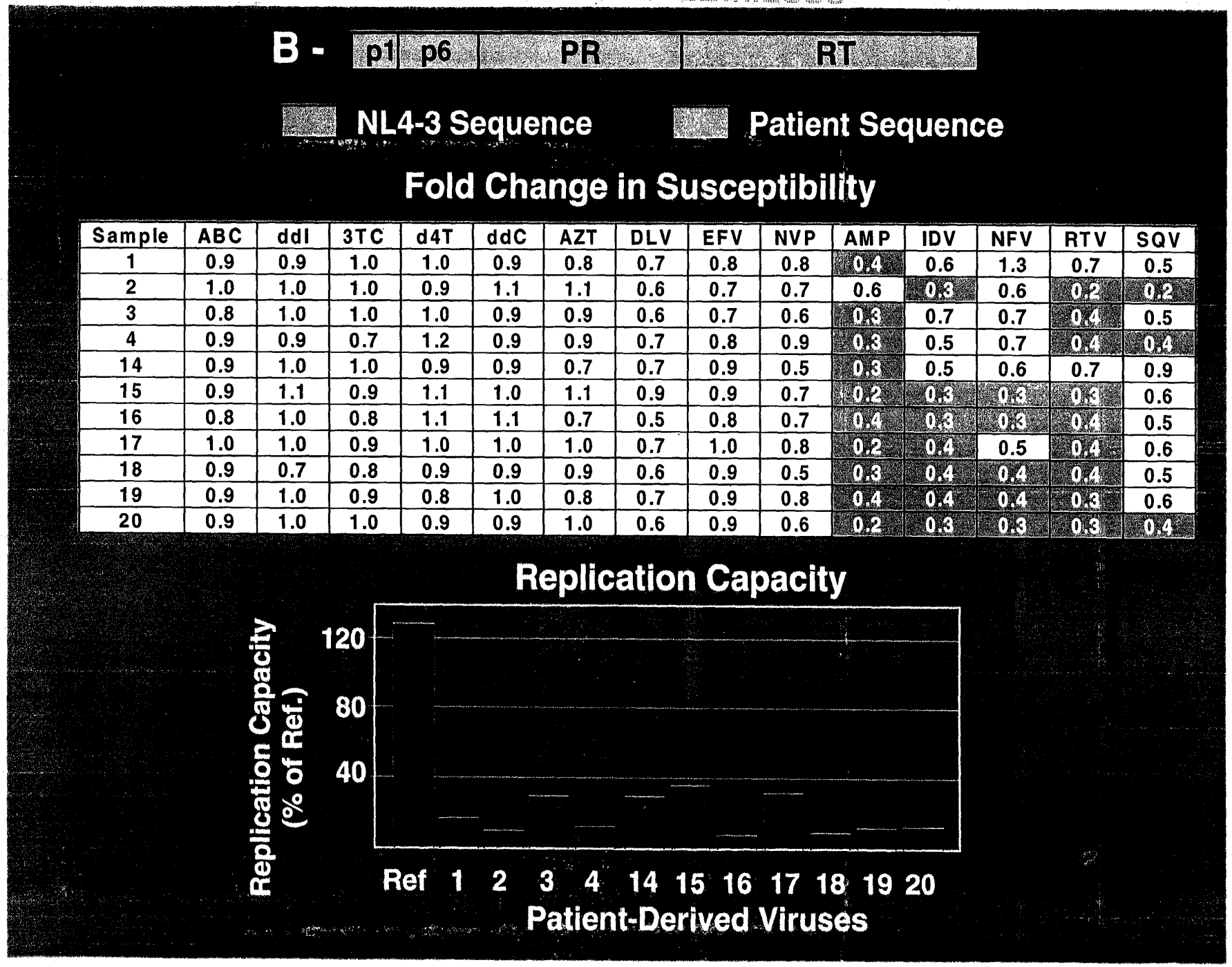
NL4-3 Sequence Patient Sequence

Fold Change in Susceptibility

Sample	ABC	ddI	3TC	d4T	ddC	ZDV	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
1	[redacted]	1.5	>300	0.8	1.5	0.8	0.7	35.8	>700	0.7	1.0	1.1	0.9	0.9
2	1.0	1.2	1.4	1.0	1.1	0.7	1.5	0.8	0.8	0.7	0.8	1.0	0.9	0.8
3	[redacted]	1.8	>300	0.9	2.1	0.7	2.1	1.1	1.4	0.6	0.9	0.9	0.7	0.4
4	[redacted]	1.8	>300	0.9	1.8	1.1	85.9	141	344	0.6	0.8	0.9	0.8	0.8
5	[redacted]	2.1	[redacted]	1.4	[redacted]	0.5	>190	>320	>700	0.5	1.0	1.1	0.7	1.0
6	[redacted]	1.4	>300	1.5	[redacted]	[redacted]	[redacted]	189	>700	0.7	0.5	0.8	0.7	0.7
7	[redacted]	[redacted]	>300	[redacted]	[redacted]	80.1	48.1	>320	>700	0.7	0.8	0.9	0.8	0.5
8														
9	1.9	1.1	>300	1.2	1.1	1.1	31.4	170	>700	0.7	0.7	1.4	0.8	0.9
10	[redacted]	1.8	>300	0.9	2.3	0.8	73.3	50	100	0.7	0.8	1.0	0.8	1.0
11	2.3	1.5	>300	0.7	1.7	0.5	35.6	130	182	0.6	1.1	1.0	1.0	0.8
12	[redacted]	1.9	>300	0.9	2.3	0.8	2.2	1.2	1.5	0.9	0.9	1.2	1.0	1.0
13	[redacted]	1.6	>300	1.0	2.1	0.4	2.1	0.8	1.2	0.8	1.0	1.0	1.0	1.0
14	[redacted]	1.8	>300	1.8	2.2	[redacted]	0.5	0.6	0.7	0.5	0.5	0.7	0.8	0.7
15	1.6	1.1	1.0	1.0	1.0	1.6	1.1	1.2	1.2	0.8	1.1	1.2	1.0	1.1
16	[redacted]	1.3	[redacted]	1.4	1.3	31	47.9	25	106	0.5	0.5	0.8	0.6	0.7
17	[redacted]	1.6	>300	0.8	2.0	2.2	12.6	33	166	0.5	0.8	0.7	0.9	0.7
18	[redacted]	1.8	>300	1.8	2.2	[redacted]	0.5	0.6	0.7	0.5	0.5	0.7	0.8	0.7
19	[redacted]	1.6	79.1	1.3	1.8	20	29	24	78	0.3	0.6	0.6	0.5	0.7
20	1.0	1.1	1.0	1.1	1.1	0.8	1.1	0.6	0.6	1.0	1.1	1.2	1.1	1.2

0 - 0.4 0.4 - 2.5 2.5 - 10 > 10

Figure 12



Fold Change in Susceptibility

Sample	ABC	ddI	3TC	d4T	ddC	AZT	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
1	0.9	0.9	1.0	1.0	0.9	0.8	0.7	0.8	0.8	0.4	0.6	1.3	0.7	0.5
2	1.0	1.0	1.0	0.9	1.1	1.1	0.6	0.7	0.7	0.6	0.3	0.6	0.2	0.2
3	0.8	1.0	1.0	1.0	0.9	0.9	0.6	0.7	0.6	0.3	0.7	0.7	0.4	0.5
4	0.9	0.9	0.7	1.2	0.9	0.9	0.7	0.8	0.9	0.3	0.5	0.7	0.4	0.4
14	0.9	1.0	1.0	0.9	0.9	0.7	0.7	0.9	0.5	0.3	0.5	0.6	0.7	0.9
15	0.9	1.1	0.9	1.1	1.0	1.1	0.9	0.9	0.7	0.2	0.3	0.3	0.3	0.6
16	0.8	1.0	0.8	1.1	1.1	0.7	0.5	0.8	0.7	0.4	0.3	0.3	0.4	0.5
17	1.0	1.0	0.9	1.0	1.0	1.0	0.7	1.0	0.8	0.2	0.4	0.5	0.4	0.6
18	0.9	0.7	0.8	0.9	0.9	0.9	0.6	0.9	0.5	0.3	0.4	0.4	0.4	0.5
19	0.9	1.0	0.9	0.8	1.0	0.8	0.7	0.9	0.8	0.4	0.4	0.4	0.3	0.6
20	0.9	1.0	1.0	0.9	0.9	1.0	0.6	0.9	0.6	0.2	0.3	0.3	0.3	0.4

Replication Capacity

Figure 13

C -

NL4-3 Sequence Patient Sequence

Fold Change in Susceptibility

Sample	ABC	ddI	3TC	d4T	ddC	ZDV	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
4	0.9	1.0	0.9	0.9	0.7	0.8	1.1	0.7	0.6	0.6	0.5	0.6	0.6	0.4
15	0.9	1.1	1.0	1.0	0.9	0.8	1.6	0.8	0.8	0.5	0.4	0.4	0.4	0.3
16	0.8	1.0	0.9	1.0	0.9	0.8	1.3	0.7	0.6	0.3	0.4	0.3	0.3	0.5
18	0.9	0.9	1.0	1.0	0.8	0.7	1.1	0.7	0.5	0.2	0.4	0.2	0.2	0.7
19	1.0	1.0	1.0	1.0	0.9	0.7	1.1	0.7	0.5	0.3	0.3	0.3	0.3	0.5

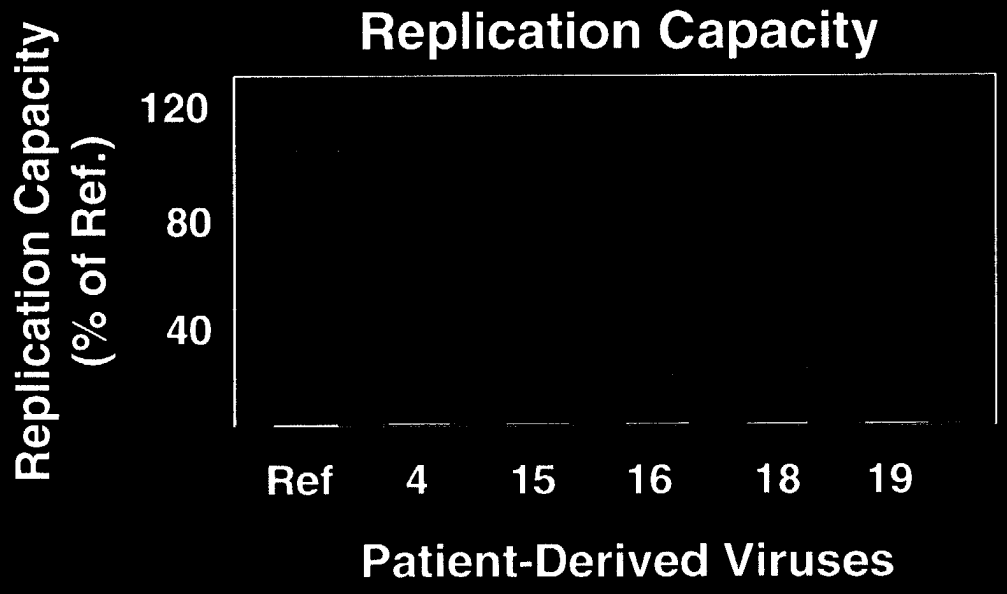


Figure 14

What Is the Role of Sequences Flanking the N-Terminus of PR?

1. The Gag Frame Encodes p1 and p6

- p6 contains the L domain (PTAPP) which is critical for virus release from the cell
- p6 is required for proper incorporation of Vpr into the virions as well as retention of pol proteins
- p6 associates with TRiC (chaperonin)

2. The Pol Frame Encodes a Transframe Protein (TFR)

TFR includes a conserved octapeptide (TFP) and p6*

- The TFP is a potent competitive inhibitor of PR in vitro
- p6* modulates PR activity

Figure 15

3. Contains Sequences and Structures Required for Frameshift

- Slippery heptamer sequence (U UUU UUA)
- Stem loop structure downstream of the frameshift site

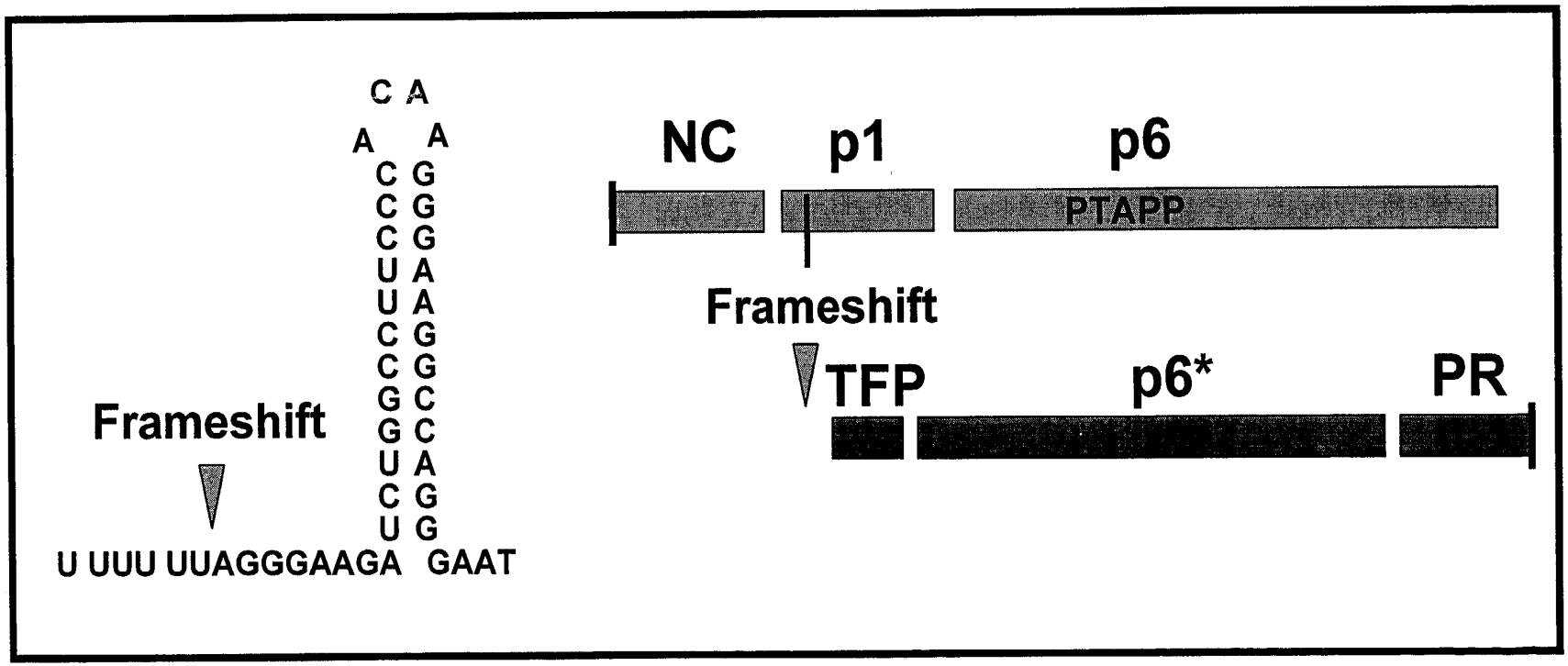


Figure 16

Genotype of Patient Derived Sequences

Gag p1 and p6

```
        ANFLGKIWPSHKGRPGNFLQSRPEPTAPPEESFRFGEETTTPSQKQEPIDKELYPLASLRSLFGNDPSSQ

I       ..........S.........N..................A......G........ST............
II      ..................V......S...A..................T...........K.......L..
III     ......  ..............N.T..................-.P.T .Q.......VT..K.......L..
IV      ..........  ........................G.....................K..........
```

Transframe Protein

```
        FFREDLAFPQ GKAREFSSEQ TRANSPTRRE LQVWGRDNNS LSEAGADRQT VSFSF

I     ........L.  ......... .......... ........S.. ......N...  ...NL
II    ....N.....  .E..KL ...  ..TI...S.. .......... .....  ....  .....
III   ....  .....  ......P...  N.......G.  ........  . P.  ......I  ..  N.
IV    ....N....    .........  ..........  ..........  .....T....  .....
```

* I to IV represent clones derived from patient sample pools that retained the HS to PI

Figure 17

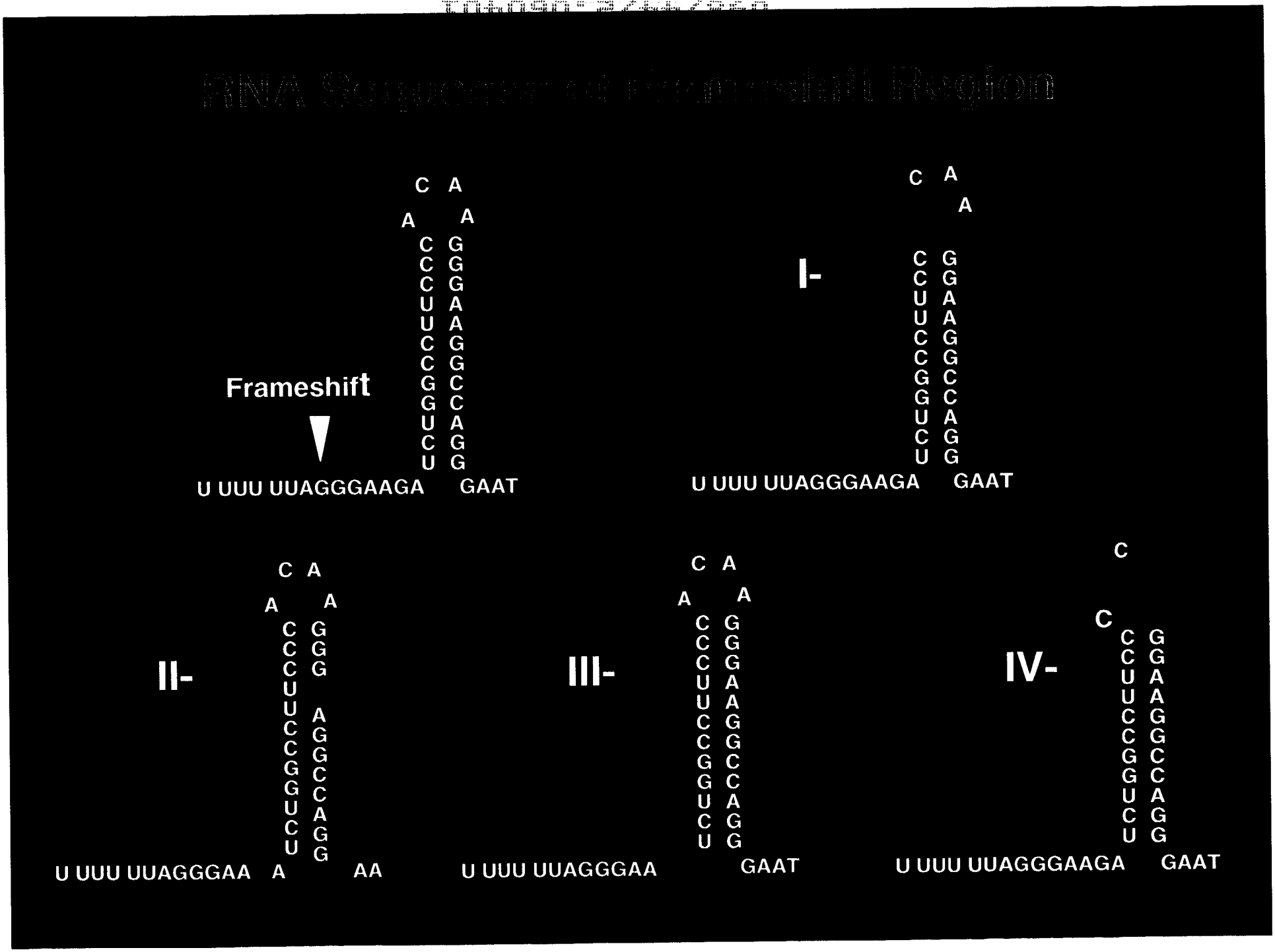
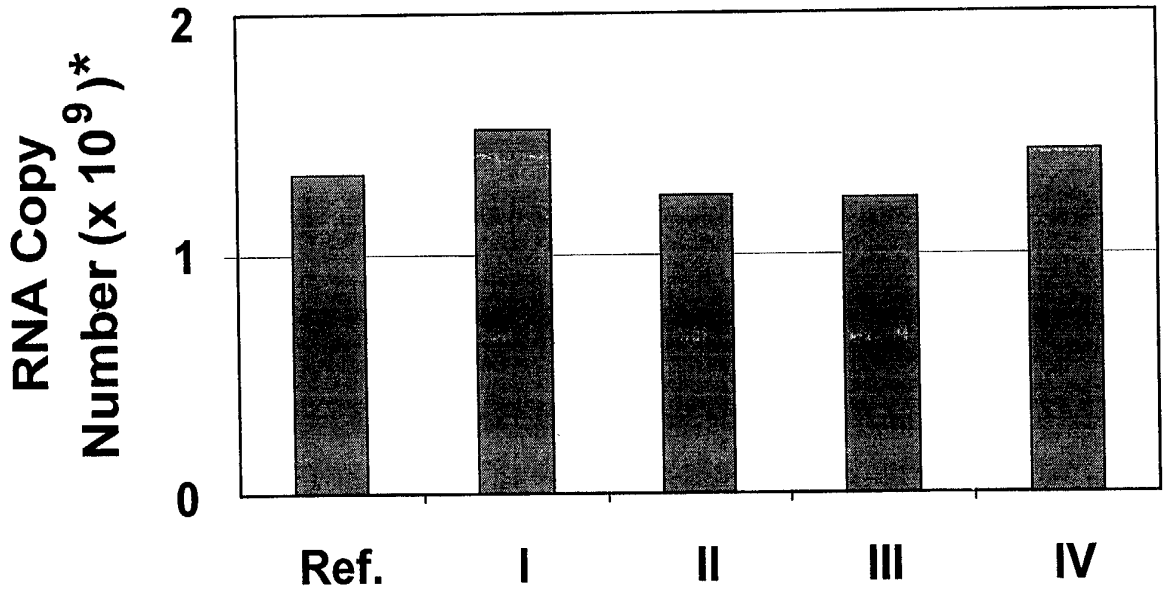

RNA Sequences at Frameshift Region
Frameshift
U UUU UUAGGGAAGA GAAT
I-
U UUU UUAGGGAAGA GAAT
II-
U UUU UUAGGGAA A AA
III-
U UUU UUAGGGAA GAAT
IV-
U UUU UUAGGGAAGA GAAT

Figure 18

Virus Release from the Cell

Quantitation of the amount of viruses produced after transfection

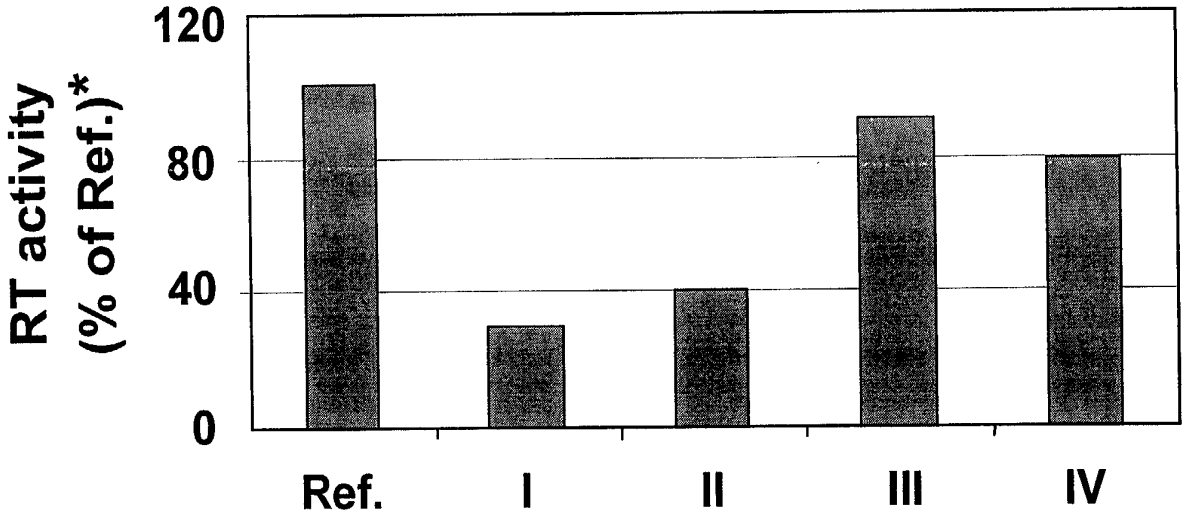


*Determined by Real Time PCR (TaqMan)

Figure 19

Pol Incorporation and/or Processing

Quantitation of virion associated reverse transcriptase activity



*Determined by Real Time PCR (TaqMan)

Figure 20

Processing of Pr55Gag in Virions

Western Blot analysis using anti-p24 antibodies

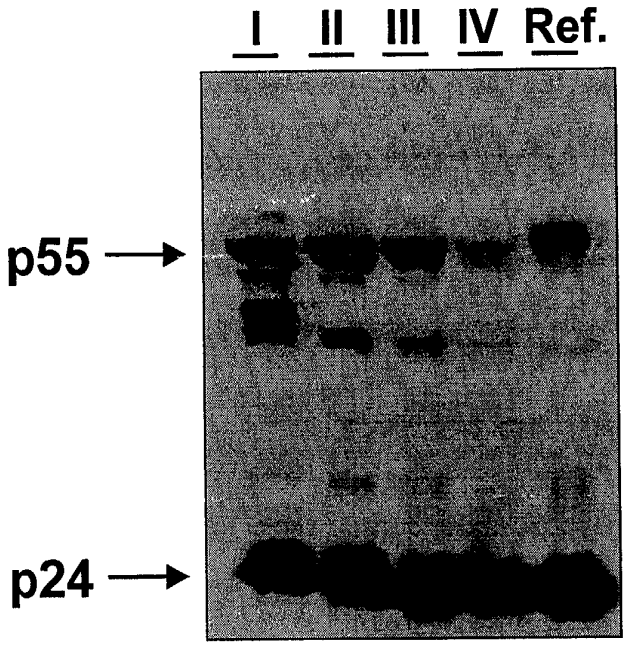


Figure 21

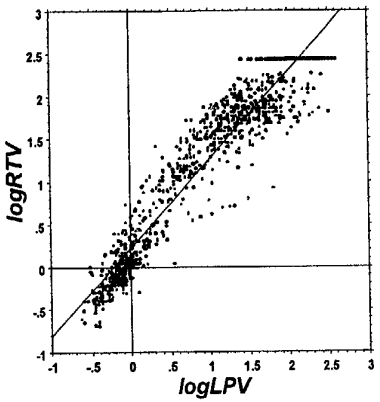
Conclusions

- HS to PIs is associated with decreased viral fitness
- In 25% of the cases analyzed in this study, the HS to PIs and decreased replication capacity was attributed to mutations in gag sequences flanking the N-terminus of PR
- Genotypic analysis revealed several unusual polymorphisms in p1-p6/TFP-p6* sequences
- Recombinant viruses carrying only the C-terminal gag sequences from patient isolates that retained the HS phenotype are released efficiently from the cell. However, analysis of the virus associated RT and PR activities suggest maturation defects

Figure 22

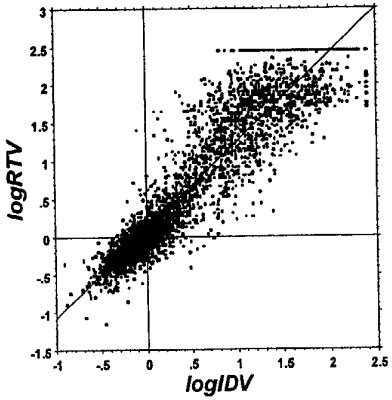
LPV vs. RTV

R^2=0.921



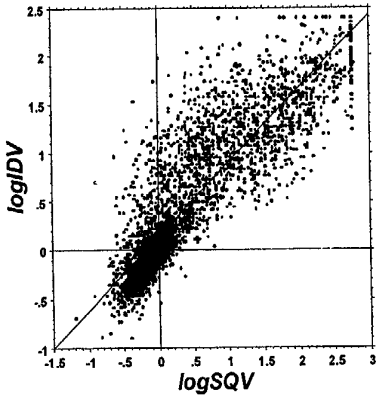
IDV vs. RTV

R^2=0.867



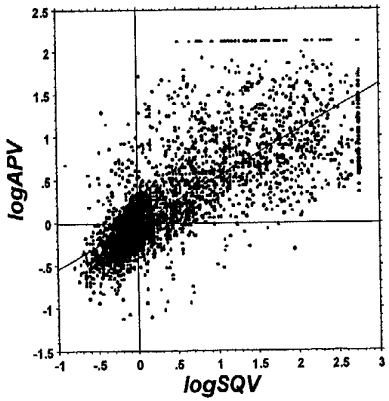
IDV vs. SQV

R^2=0.784



APV vs. SQV

R^2=0.591



These plots are examples of pairwise analysis of the extent of cross-resistance between pairs of PIs. The fold-change in IC50 vs. reference (NL4-3) of 1042 (RTV-LPV) to >3600 (other pairs) patient samples were determined using the PhenoSense assay.

Figure 23

R^2 values (sorted by drug)

PI 1	PI 2	R^2	
APV	IDV	0.675	
APV	LPV	0.777	
APV	NFV	0.544	
APV	RTV	0.737	
APV	SQV	0.591	
IDV	LPV	0.849	
IDV	NFV	0.774	
IDV	NFV	0.925	*
IDV	RTV	0.867	
IDV	SQV	0.784	
NFV	LPV	0.757	
NFV	RTV	0.696	
NFV	RTV	0.879	*
NFV	SQV	0.691	
NFV	SQV	0.801	*
RTV	LPV	0.921	
RTV	SQV	0.740	
RTV	SQV	0.871	**
SQV	LPV	0.678	

R^2 values (sorted by R^2)

PI 1	PI 2	R^2	
IDV	NFV	0.925	*
RTV	LPV	0.921	
RTV	SQV	0.871	**
NFV	RTV	0.879	*
IDV	RTV	0.867	
IDV	LPV	0.849	
NFV	SQV	0.801	*
IDV	SQV	0.784	
APV	LPV	0.777	
IDV	NFV	0.774	
NFV	LPV	0.757	
RTV	SQV	0.740	
APV	RTV	0.737	
NFV	RTV	0.696	
NFV	SQV	0.691	
SQV	LPV	0.678	
APV	IDV	0.675	
APV	SQV	0.591	
APV	NFV	0.544	

R^2 values for pairwise comparisons (all samples)

	APV	IDV	LPV	NFV	RTV	SQV
APV	1	0.675	0.777	0.544	0.737	0.591
IDV	0.675	1	0.849	0.774	0.867	0.784
LPV	0.777	0.849	1	0.757	0.921	0.678
NFV	0.544	0.774	0.757	1	0.696	0.691
RTV	0.737	0.867	0.921	0.696	1	0.740
SQV	0.591	0.784	0.678	0.691	0.740	1

<0.7
0.7-0.8
0.8-0.9
>0.9

** Excluding viruses with D30N (see Fig.4)*

*** Excluding viruses with V82AFST (see Fig.5)*

Correlation Coefficients (R^2) for all pairwise comparisons between PIs. After separating the D30N viruses in NFV comparisons (*) it can be seen that IDV, LPV, NFV and RTV have high levels of cross-resistance with each other, but that APV and SQV tend to be less cross-resistant. Removal of viruses with V82A, F, S, or T also reveals high level of cross-resistance between RTV and SQV.

Figure 24

R^2 values (sorted by drug)

PI 1	PI 2	R^2	
APV	IDV	0.675	
APV	LPV	0.777	
APV	NFV	0.544	
APV	RTV	0.737	
APV	SQV	0.591	
IDV	LPV	0.849	
IDV	NFV	0.774	
IDV	NFV	0.925	*
IDV	RTV	0.867	
IDV	SQV	0.784	
NFV	LPV	0.757	
NFV	RTV	0.696	
NFV	RTV	0.873	*
NFV	SQV	0.691	
NFV	SQV	0.801	*
RTV	LPV	0.921	
RTV	SQV	0.740	
RTV	SQV	0.880	**
SQV	LPV	0.678	

R^2 values (sorted by R^2)

PI 1	PI 2	R^2	
IDV	NFV	0.925	*
RTV	LPV	0.921	
RTV	SQV	0.880	**
NFV	RTV	0.873	*
IDV	RTV	0.867	
IDV	LPV	0.849	
NFV	SQV	0.801	*
IDV	SQV	0.784	
APV	LPV	0.777	
IDV	NFV	0.774	
NFV	LPV	0.757	
RTV	SQV	0.740	
APV	RTV	0.737	
NFV	RTV	0.696	
NFV	SQV	0.691	
SQV	LPV	0.678	
APV	IDV	0.675	
APV	SQV	0.591	
APV	NFV	0.544	

R^2 values for pairwise comparisons (all samples)

	APV	IDV	LPV	NFV	RTV	SQV
APV	1	0.675	0.777	0.544	0.737	0.591
IDV	0.675	1	0.849	0.774	0.867	0.784
LPV	0.777	0.849	1	0.757	0.921	0.678
NFV	0.544	0.774	0.757	1	0.696	0.691
RTV	0.737	0.867	0.921	0.696	1	0.740
SQV	0.591	0.784	0.678	0.691	0.740	1

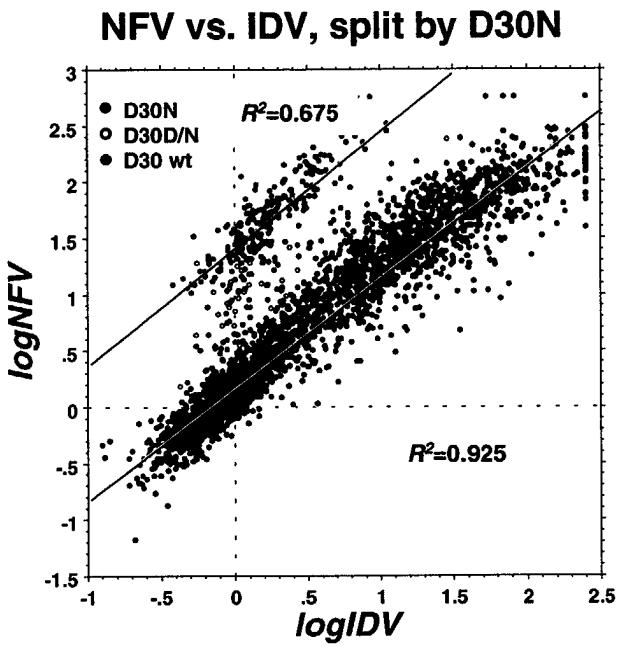
<0.7
0.7-0.8
0.8-0.9
>0.9

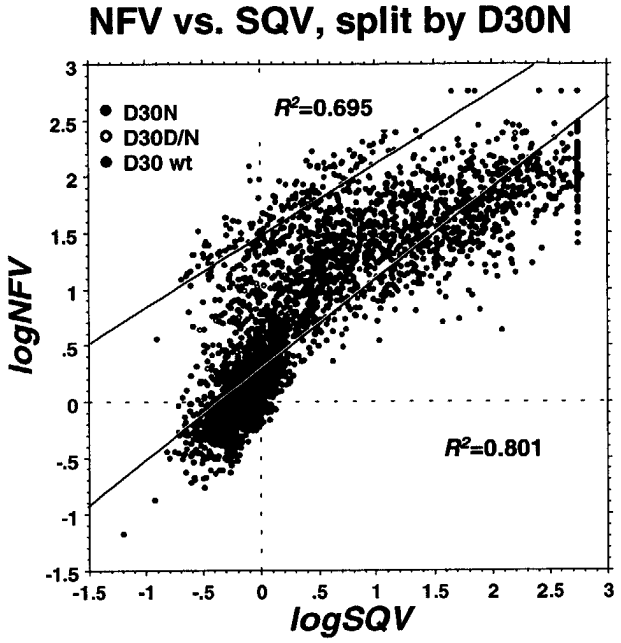
** Excluding viruses with D30N (see Fig.4)*

*** Excluding viruses with V82AFST (see Fig.5)*

Correlation Coefficients (R^2) for all pairwise comparisons between PIs. After separating the D30N viruses in NFV comparisons (*) it can be seen that IDV, LPV, NFV and RTV have high levels of cross-resistance with each other, but that APV and SQV tend to be less cross-resistant. Removal of viruses with V82A, F, S, or T also reveals high level of cross-resistance between RTV and SQV.

Figure 25

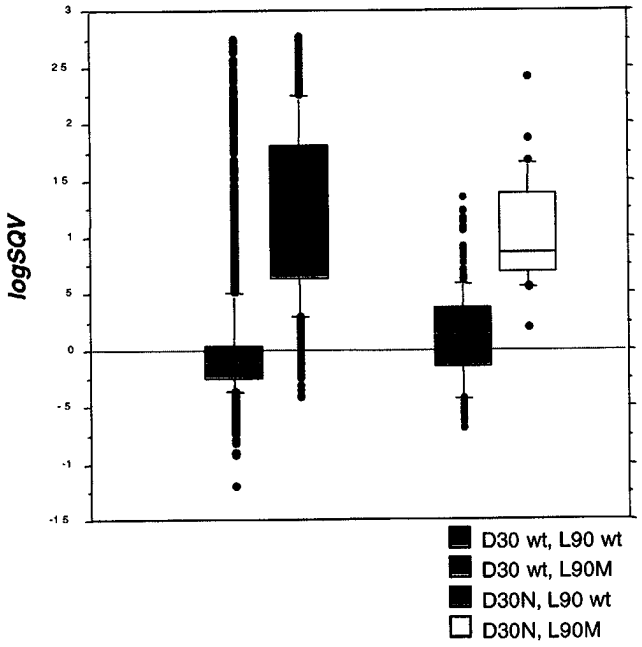




Scatter plots showing the separation of virus populations based on D30N, for IDV and NFV, or, SQV and NFV. There is still cross-resistance to IDV or SQV in D30N-containing viruses, albeit only at high levels of NFV resistance. These viruses tend to have the combination of D30N, N88D, and L90M (see next slide) The correlation between NFV and IDV in the absence of D30N is particularly striking.

Figure 26

SQV fold change +/- D30N, L90M



Phenotypes of samples containing D30N, and/or L90M, from the database (boxes contain a bar at the median and represent the 25th to 75th percentiles; the error bars represent the 10th and 90th percentiles; and the dots are the outliers.

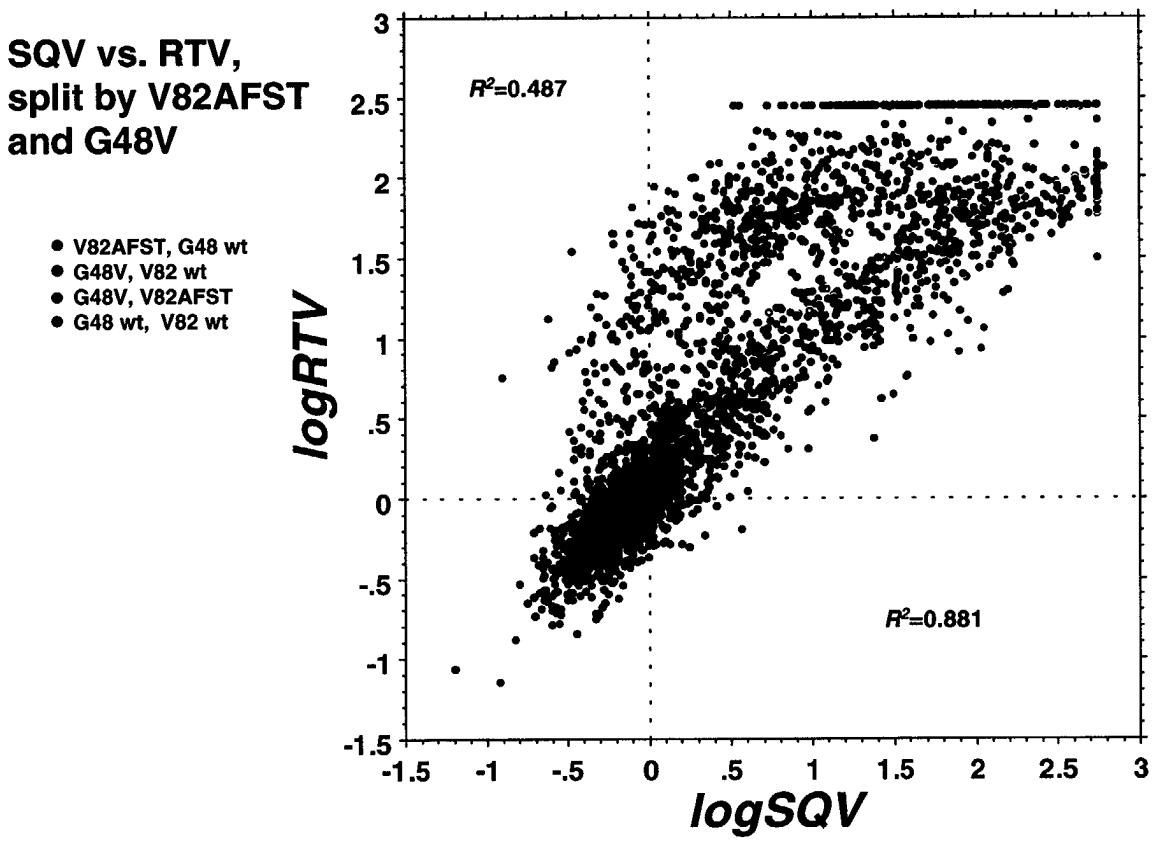
D30N/N88D/L90M: Patient samples

PR genotype (resistance-associated mutations)	Fold-change in IC_{50} vs. reference AMP	IDV	NFV	RTV	SQV
L10L/V, **D30N**, L33L/F, M36I, L63P, A71T, **N88D, L90M**	1.9	2.1	160.4	8.2	9.6
D30N, L63P, V77I, **N88D, L90M**	1.3	0.2	74.2	4.0	7.1
D30N, M36I, L63P, A71T, **N88D, L90M**	1.1	2.6	124.0	9.0	7.1
D30N, L63P, V77I, **N88D, L90M**	2.0	0.5	57.0	3.4	9.2
L10F, **D30N**, L33F, I54L, L63P, A71V, V77I, **N88D, L90M**	11.4	1.1	108.8	4.7	6.1
L10F/Y, D30N, I54L, L63P, A71T, V77I, N88D, L90M	0.4	1.3	171.4	2.1	38.1
D30N, L63P, V77I, **N88D, L90M**	2.3	0.5	32.8	0.8	3.7
L10F, D30N, L63P, A71T, V77I, N88D, L90M	2.4	5.2	217.5	10.2	11.9
L10L/R, D30N, M36I, I54I/L, L63P, A71V, N88D, L90M	1.5	5.8	140.1	16.8	21.0
D30N, M36I, I54V, L63P, A71V, **N88D, L90M**	2.3	0.5	218.5	35.0	24.3
K20K/R, **D30N**, M36I, F53F/L, I54V, L63P, A71V, **N88D, L90M**	1.2	1.7	>550	46.9	72.0
L10L/F, I13I/V, L19T, **D30N**, R41K, L63P, **N88D, L90M**	1.0	2.3	66.8	3.1	3.9
D30N, L63P, V77I, **N88D, L90M**	27.6	1.3	>550	31.2	45.3
L10F, K20T, **D30N**, L33F, M36I, M46M/I, I54L, L63P, A71V, V77I, **N88D, L90M**	1.3	1.3	35.7	1.7	3.1
D30N, L33F, L63P, A71A/T, **N88D, L90M**	1.5	0.5	73.7	0.9	5.2
D30N, L63P, V77I, **N88D, L90M**	2.2	12.0	140.4	27.0	45.8
D30N, M36I, I54V, L63P, A71V, **N88D, L90M**	>130	>250	>550	>275	257.5
L10F, K20R, **D30N**, V32V/I, L33L/F/I, M36I, M46I, I47I/V, I54I/A/M/T/V, L63P, A71V, V82V/A, **N88D, L90M**					

2.5-10 fold | >10 fold

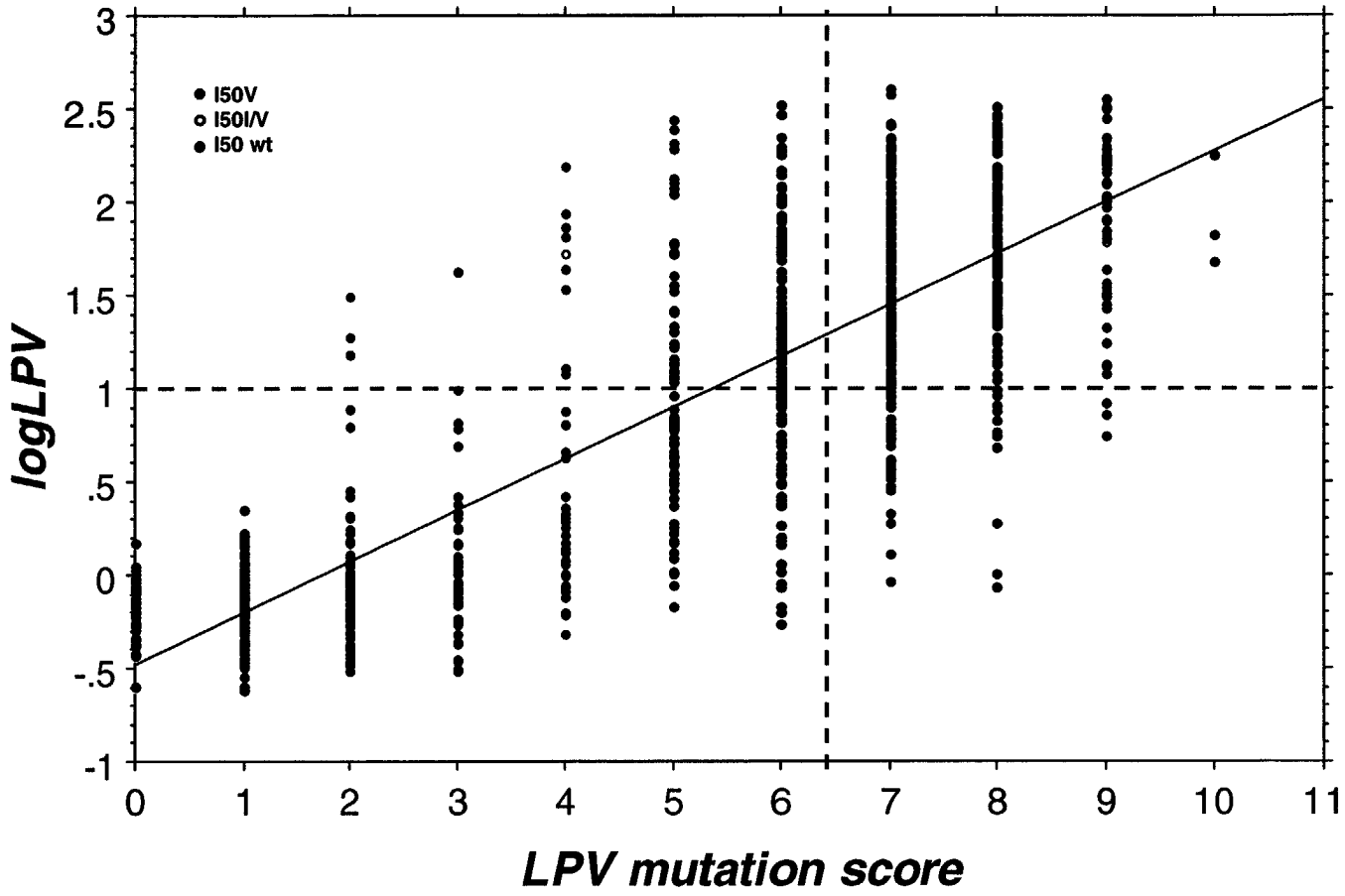
Phenotypes of samples containing D30N, N88D, and L90M. There are no mixtures detected at these sites, indicating that the mutations are linked. All have reduced susceptibility (>2.5-fold change in IC50) to NFV and SQV.

Figure 27



Scatter plot showing the separation of virus populations based on V82A, F, S, or T, for RTV and SQV. There is greater cross-resistance between RTV and SQV in viruses lacking position 82 mutations than in the population as a whole. Viruses with V82A, F, S, or T have more resistance to RTV than to SQV, unless they also have G48V (black dots)

Figure 28



Scatter plot showing the relationship between LPV susceptibility and LPV mutations score (number of mutations at positions 10, 20, 24, 46, 53, 54, 63, 71, 82, 84 and 90). Mixtures were counted as mutant and all variants at each position were considered. Clinically relevant cut points for phenotype (10-fold) and genotype (6 mutations) have been previously defined for LPV. The "false negatives" (LPV resistant with <6 mutations) contain several viruses with the I50V mutation (red dots).